Figures and figure supplements

Dissection of the interaction between the intrinsically disordered YAP protein and the transcription factor TEAD

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Figure 1. Structure of the YAP:TEAD complex. The $\alpha$-helix (A) and $\Omega$-loop (B) binding interfaces. The surface of TEAD is represented and green ribbons indicate the $\alpha$-helix (A, region 61–73) or the $\Omega$-loop (B, region 85–99) of YAP. The different YAP residues that have been mutated are indicated. Interactions between hYAP Arg89 and TEAD (C) and between hYAP Ser94 and TEAD (D). The hydrogen bonds are represented by dotted purple lines. The TEAD $\alpha$-helix (E) and $\Omega$-loop (F) binding pockets. TEAD and YAP are represented by gray and green ribbons, respectively. The mutated TEAD residues for which a $K_d^{eq}$ has been measured are represented in cyan. These figures are drawn from the pdb structure 3KYS (Li et al., 2010). TEAD residues are labeled according to hTEAD4 primary sequence.

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Figure 2. Effect of the hTEAD4 Asp272Ala mutation in cells. (A) Co-immunoprecipitation: N-terminally V5-tagged hTEAD4 (wild-type (wt) or Asp272Ala mutant) were co-transfected with wt hYAP into HEK293FT cells. YAP was immunoprecipitated, and co-immunoprecipitated nV5-TEAD4 was determined by anti-V5 Western Blot. (B) MCAT_Luc reporter assay: N-terminally V5-tagged TEAD4 (wt or Asp272Ala mutant) were co-transfected with wt hYAP into the HEK293::MCAT_Luc reporter model. Resazurin-normalized luciferase activity was measured and is plotted as fold induction over baseline. YAP and nV5-TEAD expression levels were determined in parallel by Western Blot. The expression level of hTEAD4 Asp272Ala mutant versus wt hTEAD4 was quantified by Image J software to be reduced by a factor of approx. 1.6, indicating that the approximate fourfold reduction in MCAT_Luc reporter signal cannot be solely attributed to expression differences, but does truly reflect a reduced activity of the hTEAD4 Asp272Ala mutant.

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Figure 2—figure supplement 1. Analysis of the structure of the unbound hTEAD4 Asp272Ala mutant protein by Circular Dichroism (CD). Proteins were dialyzed in 20 mM phosphate buffer pH 7.4, 100 mM KF, 0.25 mM TCEP and diluted in this buffer to 0.2 mg.mL\(^{-1}\). CD spectra were recorded on a J-815 spectropolarimeter (Jasco (France); 1 mm path length quartz cell, ‘standard’ sensitivity, 0.5 nm bandwidth, 10 nm.min\(^{-1}\) scanning, 2 s digital integration time, 1 nm step resolution). The measuring chamber was maintained in nitrogen (17 L.min\(^{-1}\)). Each spectrum was recorded as an average of 4 scans to reduce noise. After baseline correction, the mean residue ellipticity was calculated \(\theta_{\text{MRW},\lambda} = \text{MRW} \theta_\lambda / 10 \cdot \text{d.c} \), where MRW is the mean residue weight (MRW = M/(N-1)), M is the molecular mass of the protein, N is the number of amino acids in the protein, \(\theta_\lambda\) is the observed ellipticity (degrees) at wavelength \(\lambda\), d is the path length (cm) of the cell, and c is the protein concentration (g.mL\(^{-1}\)). The figure represents the CD spectra of wt (white circles) and Asp272Ala (black circles) hTEAD4\(^{217-434}\).

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Figure 3. Coupling energies at the $\alpha$-helix and $\Omega$-loop binding pockets. (A) $\alpha$-helix interface. (B) $\Omega$-loop interface. The gray and green ribbons represent the main polypeptide chain of TEAD and YAP, respectively. The $C_{\alpha}$ of the mutated amino acids are represented by spheres. The coupling energies ($\Delta G_{\text{int}}$) between the different residues are symbolized by lines. Yellow lines: $-1 \text{ kcal/mol} < \Delta G_{\text{int}} < -0.5 \text{ kcal/mol}$. Red lines: $\Delta G_{\text{int}} < -1 \text{ kcal/mol}$.

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Figure 4. Double mutant cycle for the hYAP Arg89: hTEAD4 Asp272 interaction. (A) The binding energies (kcal/mol) measured for the different pairwise interactions are indicated in boxes, and the differences between these binding energies (ΔΔG<sub>1-4</sub>) are shown. The coupling energy (ΔΔG<sub>int</sub> = ΔG<sub>wt:wt</sub> + ΔG<sub>R89A:D272A</sub> – ΔG<sub>R89A:wt</sub> – ΔG<sub>wt:D272A</sub>) is indicated (kcal/mol). Standard errors (SE) are given. (B) Representative sensorgrams of the Surface Plasmon Resonance experiments carried out to establish the double mutant cycle presented on A. wt or Asp272Ala hTEAD4 were immobilized on sensor chips and the binding of different concentrations of analyte (hYAP wt or Arg89Ala) was measured. K<sub>d</sub> values were measured at equilibrium (K<sub>d</sub><sub>eq</sub>). The contact time in the experiments was varied according to the affinity of each of the YAP proteins. (C) Binding isotherms obtained from the sensorgrams presented on B. The experiments were fitted with the Biacore T200 evaluation software using a one site binding with background model. The K<sub>d</sub><sub>eq</sub> values and the standard error (SE) from the fit are indicated.

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The following source data is available for figure 4:

Source data 1. The double mutant cycles established in this study.
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Figure 4—figure supplement 1. Position of hYAP Phe69 and hTEAD4 Phe337 at the α-helix binding pocket. The α-helix (region 61–73) of YAP is represented by a green ribbon and hYAP Phe69 is indicated. The surface of TEAD is in gray and hTEAD4 Phe337 in orange. This figure was drawn from the pdb structure 3KYS (Li et al., 2010). DOI: 10.7554/eLife.25068.011