Exploiting *Forster Resonance Energy Transfer* (FRET) in the characterization of YAP:TEAD complex disruption

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ABSTRACT

The development of chemical tools to interrogate biological systems is pivotal in Drug Discovery. In particular, the knowledge of the chemical properties of biomolecules can be exploited to study proteins structural features and protein-protein interactions (PPI). In our case study, we have exploited different chemical biological tools to characterize the human transcriptional enhancer associated domain (hTEAD4) to screen disrupters of YAP:TEAD complex.

YAP:TEAD complex is the endpoint of the Hippo signalling pathway, whose deregulation is recognised as a solid cancer hallmark and a metastasis trigger. When the pathway is active, its coactivator YAP1 is phosphorylated and retained in the cytosol. When the pathway is inactive, YAP1 is de-phosphorylated and migrates into the nucleus to bind to TEAD1-4. The complex formation promotes the transcription of genes involved in cell survival and proliferation [1]. So far, the crystal structure of the complex revealed that the two proteins interact with three different interfaces; interface 3 plays a key role in the complex formation. Also, recent thermodynamic and hotspot-mutation studies have validated the YAP:TEAD interface 3 as a drug target for the development of innovative anticancer drugs [2]. Little knowledge about simplified assays to screen disrupters is responsible for a slow hit identification achievement. Aiming to set up an assay suitable for Ligand:TEAD binding interaction studies, a simplified model of interaction at interface 3 has been designed. YAP:TEAD structure-based visual inspection exploited hTEAD4 binding domain (YBD, aa 217-434) and a short YAP-mimicking peptide conjugated with a fluorescent probe [3]. hTEAD4 YBD cysteines' reactivity was investigated using a thiol/disulphide small compound library. Cys-335 is <15 Å away from interface 3, and it showed a selective reactivity towards maleimide moiety. For this feature, the protein was conjugated with fluorescein or tetramethylrhodamine-5-maleimide to exploit a FRET (Förster resonance energy transfer) assay with the properly labelled YAP mimicking peptide ligand. More compounds were studied as interactor of TEAD binding domain. The addition of the peptide to the protein showed a concentration dependent effect. The explorative experiments performed showed the feasibility of the Kd measurements and the need for further optimization studies.

In conclusion, a promising tool to screen compound targeting interface 3 of YAP:hTEAD complex has been set up exploiting protein reactivity.

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