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TOWARDS THE DESIGN OF SELECTIVE LIGANDS OF  
TEADS C-TERMINAL DOMAIN

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## **Abstract**

The Hippo signaling pathway plays a fundamental role in the control of organ growth, cell proliferation and stem cell characters. TEADs are the main transcriptional output regulators of the Hippo signaling pathway and bind to YAP and TAZ coactivators. TEAD1-4 are expressed differently depending on the tissue, developmental level and can be overexpressed in certain pathologies. TEAD ligands mainly target the internal pocket of the C-terminal domain of TEAD and the first selective ligands for TEAD1 and 3 have been recently reported. In this paper, we focus on the topographic homology of the TEAD C-terminal domain both externally and in the internal pocket to highlight the possibility of rationally designing ligands selective for one of the TEAD family members. We identified a novel TEAD2-specific pocket and reported its first ligand. Finally, alphafold2 models of full-length TEADs suggest TEAD autoregulation and emphasize the importance of the interface 2.

## I. Introduction

### A. Hippo pathway

The evolutional conserved Hippo pathway, firstly discovered in *Drosophila* in 1995,<sup>1</sup> controls organ size and cell differentiation through the regulation of cell proliferation and apoptosis. Growing interest in the Hippo pathway has been firstly driven by its deregulation in many diverse cancers (as reported in many reviews),<sup>2,3</sup> then found interest in regenerative medicine<sup>4-6</sup> to finally emerged in the field of neurodegenerative diseases.<sup>7,8</sup> The Hippo pathway is mainly composed of a series of kinases which after stimulation by upstream signals causes the YAP (Yes-Associated Protein) /TAZ (transcriptional co-activator with PDZ-binding motif, also known as WWTR1 (WW domain-containing transcription regulator protein 1) phosphorylation to remain in the cytoplasm, eventually leading to its capture and degradation. When the Hippo pathway is inactivated, unphosphorylated YAP/TAZ enters the nucleus and promote downstream genes expression through the formation of transcriptional complexes with other transcription factors such as RUNX1/2, TBX5,<sup>9</sup> and specially TEAD1-4 transcription factors which are considered as the most important output of the Hippo pathway.

## B. Physiological pan-TEAD pathway

TEAD family has Hippo-dependent coactivators (YAP and TAZ) but also Hippo-independent coactivators (VGLL1-4) and newly discovered interactors (FAM181 proteins).

YAP and TAZ (transcriptional co-activator with PDZ-binding motif, also known as WWTR1) bind to TEAD1-4 to drive expression of target genes such as CTGF, Cyr61 or Axl (cell proliferation), Survivin (Birc-5) (inhibition of apoptosis), Sox2, Nanog and Oct4 (stemness, pluripotency). Besides YAP/TAZ, the most studied coactivators of TEAD1-4 transcriptional activity, the Vestigial-like (VGLL) protein family, which consists in four members (VGLL1-4), has been shown to interact with TEAD to regulate gene expression<sup>10</sup>.

Although VGLL1-4 bind to the YAP-TAZ binding domain of TEAD1-4, VGLL1-3 and VGLL4 have distinct molecular functions and only VGLL4 has been reported to inhibit YAP/TAZ-TEAD interactions.

A third family of TEAD interactor has been recently identified. FAM181 genes constitute a novel gene family<sup>11</sup> that is conserved among vertebrates with two paralogs, namely FAM181A and FAM81B, per species. Both genes display highly dynamic and specific expression patterns (most prominently in neural tissues)

during murine embryonic development<sup>12</sup>. These last proteins also interact with TEAD in the same C-terminal domain than YAP, TAZ and VGLLs.

Mammals express four TEAD genes located on four different chromosomes. TEADs are broadly expressed in almost all tissues with a specific pattern that indicates tissue-specific roles for each TEAD members. TEADs play important roles in development (cardiogenesis (TEAD1), neural crest (TEAD2) and notochord (TEAD1/2) development, cell lineage fate determination<sup>13</sup>) but each TEAD has tissue specific expression (TEAD1: schwann cell development, smooth muscle cell proliferation and differentiation, angiogenesis stimulation in endothelial cells; TEAD2: mouse embryonic stem cell self-renewal; TEAD4: myogenic differentiation). Recently, TEAD1 and TEAD3 were found to regulate human epidermal proliferation<sup>14</sup> while TEAD3 mediates cardiac lineage formation from human-induced pluripotent stem cells.<sup>15</sup>

### C. Pathologies with specific TEAD dysregulation

Dysregulation of the Hippo pathway may occur in many diseases, including cancers, neurodegenerative diseases and rare diseases. Almost all the upstream regulators may be dysregulated. Here, we specifically focus on dysregulation of TEADs.

A specific mutation of TEAD1 (Y421H) is associated with Sveinsson's chorioretinal atrophy, a rare genetic disorder that results in degeneration of the choroid and retina.<sup>16</sup> TEAD1 and YAP regulate ballooning cell death in Htt17Q expressing primary mice neurons (a Huntington disease cellular model).<sup>17</sup> YAP and TEAD1 are specifically expressed in Müller cells and are upregulated following retinal degeneration.<sup>18</sup>

In cancers, TEAD1 is mainly implicated in prostate, cervical and breast cancers.<sup>19,20</sup> TEAD2 has been reported to be a novel prognostic factor for hepatocellular carcinoma<sup>21</sup> and its expression is increased during epithelial-mesenchymal transition in mammary gland epithelial cells and breast cancer cells,<sup>22</sup> and emerged as an independent prognostic factor in pancreatic ductal adenocarcinoma.<sup>23</sup> In glioblastoma, neurofibromatosis 2 controls the invasiveness through YAP-TEAD2-dependent expression of Cyr61.<sup>24</sup> TEAD4 expression significantly increases in colorectal cancer<sup>25</sup> and in hepatoblastoma<sup>26</sup> and elevated levels of a splicing isoform of TEAD4 associated with an increased rate of survival was also reported in cancer patients.<sup>27</sup>

Amongst the different strategies to combat YAP and TAZ in cancers that have been recently reviewed,<sup>28</sup> targeting YAP/TAZ-TEAD complexes (group II compounds) by TEAD ligands have yielded to published or patented pan-TEAD

inhibitors<sup>29</sup> but subtle structural differences in the TEAD internal pocket have already been suggested to give selective TEAD inhibitors.<sup>30</sup> Creating selective TEAD ligands would find interest in diseases where one TEAD is overexpressed but also in deciphering the exact role of each TEAD member in healthy or disease cellular models.

Here, starting from the newly available structures of hTEADx from AlphaFold2, we reanalyzed the highly conserved but non-identical C-terminal domains of the four members of the TEAD family. Two main strategies of TEAD targeting are: disrupting the protein-protein interaction with their activators or modulating the acylation of their internal pocket. Facing the growing evidence of major divergence of function between the TEAD family members, we focused on the differences that may give rise to selectivity: (i) the known internal pocket and the acylation, (ii) the protein-protein interface with the yet-unexplored flexible loop of hTEAD2 (loop A) and the non-conserved loop that is close to both ends of the C-terminal domain (loop B). We described here a ligand of this new specific TEAD2 loop A which will pave the way to the discovery of a selective ligand of hTEAD2. Ligands displaying selectivity over some of the TEAD family members have already been described, albeit in small number and solely toward the internal pocket such as VT103 (**16**), the first selective inhibitor of hTEAD1 auto-palmitoylation patented by Vivace Therapeutics<sup>31-33</sup> and DC-TEAD3in03 (**17**), a



selective inhibitor of hTEAD3<sup>34</sup>. We thus performed a docking study to explore the structural determinants that could underlie such specificity, using Alphafold2 and in-house model developed to take account of the plasticity of the internal pocket where the internal cavity is enlarged by a non-fatty acid ligand.

Our conclusion led us to note some discrepancies regarding the paradigm of TEAD activation by its protein partners and once again, using the Alphafold2 structures and some recent results, we were able to formulate a new hypothesis that allow further development of TEAD modulators.

## II. TEAD structures & functions

### A. TEAD-activators protein-protein interfaces

Entry	TEAD	Resolution	Molecule in the internal pocket	External partners	PDB code Ref
1	hTEAD1 <sup>209-426</sup>	2.80 Å	Palmitate	YAP <sup>50-171</sup>	3KYS <sup>35</sup>
2	hTEAD1 <sup>209-426</sup>	2.20 Å	Palmitate	Engineered YAP	4RE1 <sup>63</sup>
3	hTEAD1 <sup>210-426</sup>	1.70 Å	Palmitate	/	6IM5 <sup>67</sup>
4	hTEAD1 <sup>208-425</sup>	2.30 Å	Myristate	YAP <sup>60-100</sup>	6HIL <sup>47</sup>
5	hTEAD1 <sup>212-426</sup>	3.50 Å	K-975 (1)	/	7CMM <sup>57</sup>
6	hTEAD2 <sup>218-446</sup>	2.00 Å	Palmitate	/	5EMV <sup>43</sup>
7	hTEAD2 <sup>217-453</sup>	2.05 Å	Palmitate	/	5HGU <sup>44</sup>
8	hTEAD2 <sup>217-447</sup>	2.00 Å	Palmitate	/	3L15 <sup>36</sup>
9	hTEAD2 <sup>217-447</sup>	2.30 Å	Flufenamate(2)	/	5DQ8 <sup>52</sup>
10	hTEAD2 <sup>217-447</sup>	2.18 Å	Bromofenamate (3)	/	5DQE <sup>52</sup>
11	hTEAD2 <sup>217-447</sup>	2.32 Å	MGH-CP1 (4)	/	6CDY <sup>53</sup>
12	hTEAD2 <sup>217-447</sup>	1.54 Å	Pyrrolidin-2-one (5)	/	6UYB <sup>56</sup>
13	hTEAD2 <sup>217-447</sup>	1.66 Å	(6)	/	6UYC <sup>56</sup>
14	hTEAD2 <sup>217-447</sup>	2.11 Å	(7)	/	6VAH <sup>55</sup>
15	hTEAD2 <sup>217-447</sup>	2.43 Å	TED-347 (8)	/	6E5G <sup>58</sup>
16	hTEAD2 <sup>217-447</sup>	2.20 Å	Myristate	Pyrazole (10)	6S66 <sup>66</sup>
17	hTEAD2 <sup>217-447</sup>	2.00 Å	Myristate	Pyrazole (11)	6S60 <sup>66</sup>
18	hTEAD2 <sup>217-447</sup>	2.10 Å	Myristate	Pyrazole (12)	6S6J <sup>66</sup>
19	hTEAD2 <sup>217-447</sup>	2.15 Å	Myristate	Pyrazole (13)	6S69 <sup>66</sup>
20	hTEAD2 <sup>217-447</sup>	2.22 Å	Myristate	Pyrazole (14)	6S64 <sup>66</sup>
21	hTEAD3 <sup>219-435</sup>	2.55 Å	Palmitate	/	5EMW <sup>43</sup>
22	hTEAD3 <sup>219-435</sup>	2.60 Å	VT105 (9)	/	7CNL <sup>33</sup>
23	hTEAD4 <sup>217-434</sup>	1.95 Å	Myristate	YAP <sup>60-100</sup>	5OAQ <sup>48</sup>
24	hTEAD4 <sup>216-434</sup>	1.85 Å	Myristate	YAP <sup>60-100</sup>	6GE3 <sup>46</sup>
25	hTEAD4 <sup>216-434</sup> (E263A)	1.97 Å	Myristate	YAP <sup>60-100</sup>	6GE4 <sup>46</sup>
26	hTEAD4 <sup>216-434</sup> (Y429F)	2.05 Å	Myristate	YAP <sup>60-100</sup>	6GE5 <sup>46</sup>

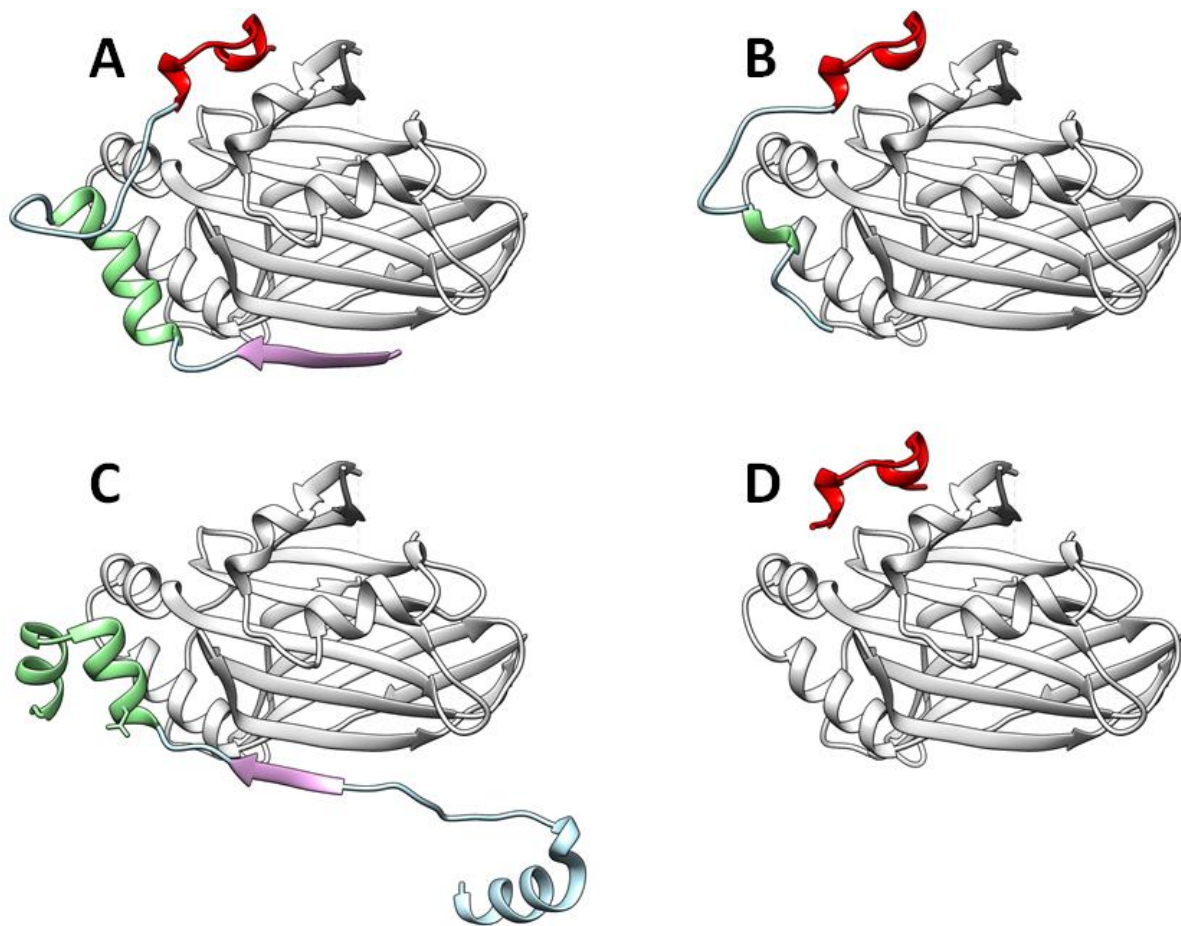
27	hTEAD4 <sup>216-434</sup> (E263A/Y429F)	1.80 Å	Myristate	YAP <sup>60-100</sup>	6GE6 <sup>46</sup>
28	hTEAD4 <sup>216-434</sup>	1.70 Å	Myristate	YAP <sup>59-99</sup> (S94A)	6GEC <sup>46</sup>
29	hTEAD4 <sup>216-434</sup> (E263A)	1.96 Å	Myristate	YAP <sup>59-99</sup> (S94A)	6GEE <sup>46</sup>
30	hTEAD4 <sup>216-434</sup> (Y429F)	2.23 Å	Myristate	YAP <sup>59-99</sup> (S94A)	6GEG <sup>46</sup>
31	hTEAD4 <sup>216-434</sup> (E263A/Y429F)	1.65 Å	Myristate	YAP <sup>60-100</sup> (S94A)	6GEI <sup>46</sup>
32	hTEAD4 <sup>216-434</sup> (Y429F)	2.28 Å	Myristate	YAP <sup>60-100</sup>	6GEK <sup>46</sup>
33	hTEAD4 <sup>216-434</sup>	1.65 Å	Myristate	YAP <sup>59-99</sup>	6HIK <sup>47</sup>
34	hTEAD4 <sup>217-434</sup>	2.01 Å	Myristate	Peptide	6Q36 <sup>64</sup>
35	hTEAD4 <sup>217-434</sup>	2.10 Å	Myristate	YAP <sup>60-100</sup>	6Q2X <sup>64</sup>
36	hTEAD4 <sup>217-434</sup>	1.65 Å	Myristate	FAM181A	6SEN <sup>42</sup>
37	hTEAD4 <sup>217-434</sup>	2.55 Å	Myristate	FAM181B	6SEO <sup>42</sup>
38	mTEAD4 <sup>209-427</sup>	2.555 Å	Myristate	Novel FAM181A	6L9F
39	mTEAD4 <sup>210-427</sup>	2.90 Å	Myristate	TAZ <sup>24-57</sup>	5GN0 <sup>38</sup>
40	mTEAD4 <sup>210-427</sup>	3.00 Å	Myristate	YAP <sup>47-85</sup>	3JUA <sup>37</sup>
41	mTEAD4 <sup>209-427</sup>	2.90 Å	Myristate	VGLL4 <sup>203-256</sup>	4LN0 <sup>40</sup>
42	mTEAD4 <sup>210-427</sup>	2.22 Å	Myristate	Fragment ( <b>15</b> )	5XJD <sup>65</sup>
43	mTEAD4 <sup>209-427</sup>	1.30 Å	Myristate	Mimetic VGLL4	6SBA <sup>41</sup>
44	mTEAD4 <sup>210-427</sup>	2.74 Å	Myristate	VGLL1 <sup>20-51</sup>	5Z2Q <sup>39</sup>
45	Sd <sup>222-440</sup>	1.85 Å	Myristate	Vg <sup>298-337</sup>	6Y20 <sup>76</sup>

*Table 1. Available structures of C-terminal domain of TEAD resolved by X-ray diffraction*

NMR and crystallographic structures (see Table 1) of YAP-TEAD complexes were reported in 2010 by several groups<sup>35-37</sup> while mTAZ-mTEAD crystallographic structure was reported in 2017.<sup>38</sup> Two structures have been reported for mVGLL-mTEAD4 interactions<sup>39,40</sup> and very recently a short peptide mimicking mVGLL4

was designed and crystallized with mTEAD4 C-terminal domain.<sup>41</sup> Finally, FAM181A and B peptides were crystallized with hTEAD4.<sup>42</sup>

All these fragments of proteins (YAP, TAZ, VGLL and FAM) binds to TEAD C-terminal domain at its external surface (interfaces 1, 2 and 3) (Figure 1 and Table 2).



*Figure 1. The different TEAD complexes: (A) with YAP (PDB code: 3KYS); (B) with TAZ (PDB code: 5GNO); (C) with VGLL4 (PDB code: 4LN0); (D) with FAM181A (PDB code: 6SEN); TEAD is in light grey, the  $\Omega$  loops at interface 3 are in red, the  $\alpha$  helices at interface 2 are in green and light blue and the  $\beta$  sheets*

at interface 1 are in purple. All structural figures were generated with Chimera 1.10.2 (UCSF).<sup>80</sup>

The interaction of these proteins with TEAD involves the three same interfaces, with YAP, TAZ and VGLL2 binding to the three, VGLL1&3 binding to the interface 1 and 2 and the FAM181 only to the third. VGLL4 is a bit divergent, with a binding to a first TEAD monomer in the interface 1 and 2 and with a second TEAD monomer in the interface 2. All but VGLL4 are activators of TEAD, even if the involvement of FAM181 remains unclear, their main difference coming from their cellular context of effect: It appears VGLL members act mostly when the Hippo pathway is on while YAP/TAZ interact with TEAD when the Hippo pathway is off.

<b>hYAP1</b>	<sup>52</sup> HQIVHV----RGDSETDLEALF-NAVMNPKTANVPQTVPMRLRKL PDSFFK PPE <sup>100</sup>
<b>mYAP1</b>	<sup>37</sup> HQVVHV----RGDSETDLEALF-NAVMNPKTANVPQTVPMRLRKL PDSFFK PPE <sup>85</sup>
<b>hTAZ</b>	<sup>15</sup> QQVIHV----TQDLDTDLEALF-NSVMNPK----PSS-WRKKILPESFFKEP-D <sup>57</sup>
<b>mTAZ</b>	<sup>15</sup> QQVIHV----TQDLDTDLEALF-NSVMNPK----PSS-WRKKILPESFFKEP-D <sup>57</sup>
<b>mVGLL1</b>	<sup>25</sup> AGSVIFT-YFEGDINSMVDEHFSRALRNLK <sup>53</sup>
<b>mVGLL4</b>	<sup>227</sup> SVSIT----G---S-VDDHFAKALGDTWLQIKAA <sup>252</sup>
<b>hFAM181A</b>	<sup>191</sup> PMRKRQLPASFWEEP <sup>205</sup>
<b>hFAM181B</b>	<sup>220</sup> VPLRARNLPPSFFTEP <sup>235</sup>

Table 2. Sequence alignments of TEAD binding domain of YAP, TAZ, VGLLs and FAM181. Residues of the interfaces 1, 2 and 3 are highlighted in purple, green and

*red respectively. Numbering of the different proteins can be found in <http://www.uniprot.org/> (P46937, P46938, Q9GZV5, Q9EPK5, Q99NC0, Q80V24, Q8N9Y4 and A6NEQ2).*

#### B. TEAD internal pocket

In 2016, the presence of palmitate in an internal pocket of hTEAD2 or hTEAD3 was reported for the first time, which seems to increase the stability of the TEAD protein.<sup>43-47</sup> This internal pocket can also welcome a myristate and those fatty acids were found free or bonded to conserved cysteine (C380 in hTEAD2) (thioester function) or lysine residues (K357 in hTEAD2) (amide function) (Figure 2).<sup>48</sup> Since direct N-acylation of a lysine residue with palmitoyl-CoA is relatively slow, a cysteine-to-lysine transfer<sup>49-50</sup> is expected to probably occur in the pocket due to the proximity of both residues, explaining the observations made by Noland et al.<sup>43</sup> on the irreversibility of TEAD palmitoylation which was considered rather uncharacteristic.

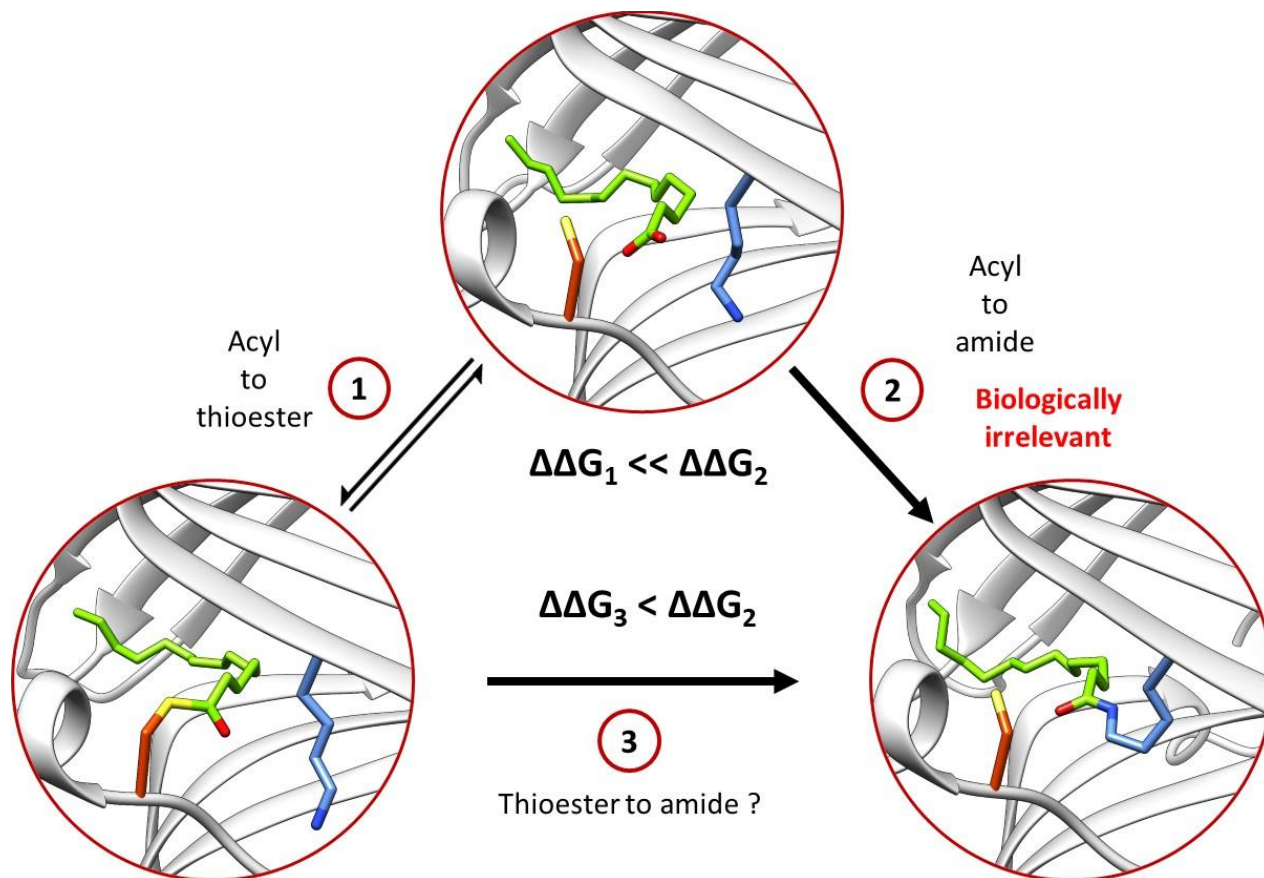


Figure 2. View of the entry of the internal pocket where the fatty acid is free (top) or bonded to conserved cysteine (left) or lysine (right)

The internal pocket can be divided into 2 sub-pockets:<sup>6</sup> a highly conserved hydrophobic sub-pocket (Figure 3 and table 3, in blue) located at the bottom which accommodates the carbon skeleton of the fatty acid which therefore interacts with these amino acids through Van der Waals interactions and a hydrophilic sub-pocket (Figure 3 and table 3, in purple) which is close to the entrance and surrounds the acid moiety of the fatty acid. This carboxylic function interacts by hydrogen bonds, through covalent bond with conserved cysteine (in grey) and lysine residues or ionic bond with lysine residue.

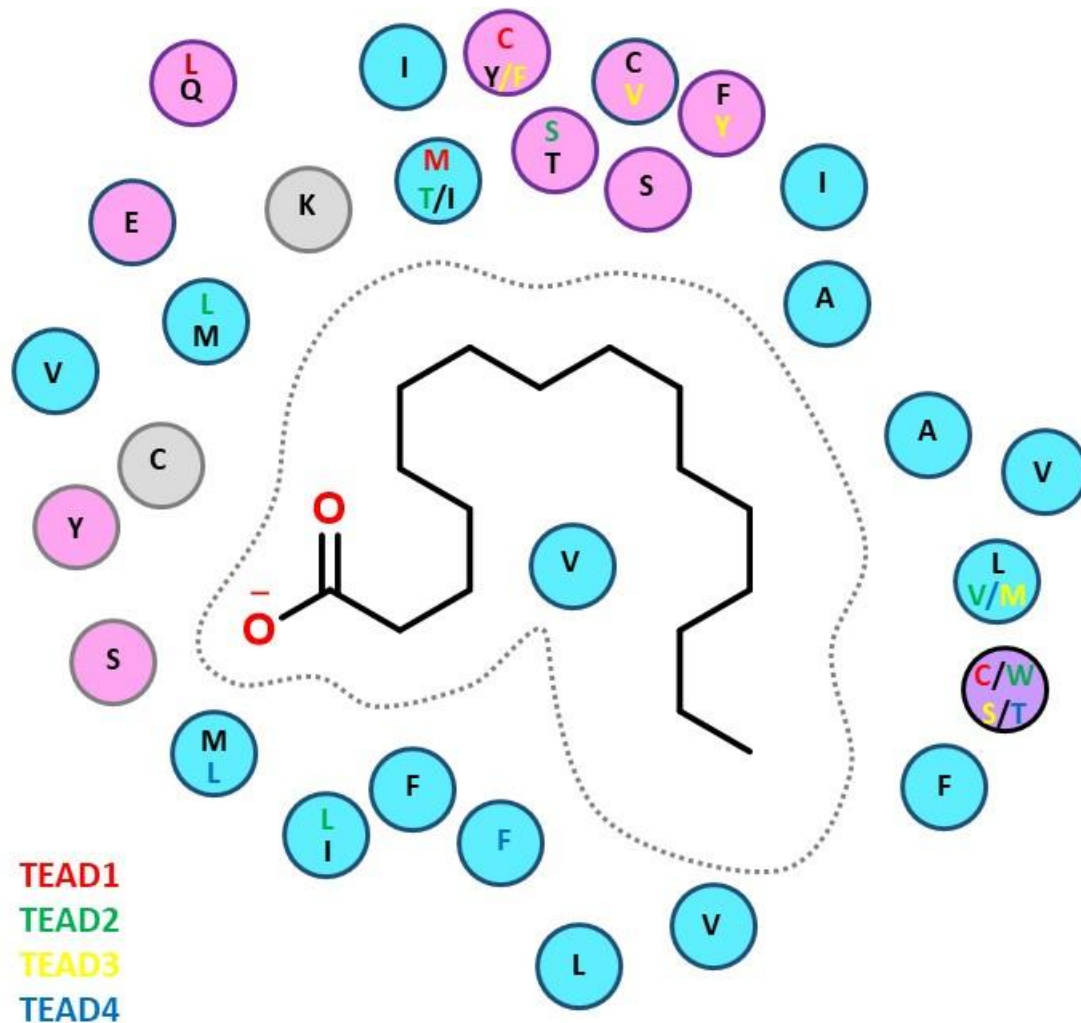


Figure 3. Hydrophobic and hydrophilic sub-pockets of the internal pocket of hTEAD2; grey circles correspond to residues that make covalent bond with fatty acid; blue circles correspond to hydrophobic residues; magenta circles correspond to hydrophilic residues; purple circle corresponds to non-conserved residues

Whereas the hydrophobic sub-pocket is highly conserved, the hydrophilic sub-pocket displays significant differences (see Figure 3 and Table 3): at the bottom we



found C405 for hTEAD1, Y426 and Y413 for hTEAD2 and hTEAD4 and F414 for hTEAD3; at the entry of the hydrophobic pocket we found Y233 for hTEAD3 and a phenylalanine residue for the other hTEADs (F221, F233 and F229 for hTEAD1, hTEAD2 and hTEAD4 respectively); finally, close to C405, hTEAD1 has L389 which is a glutamine residue (Q410, Q398 and Q397 for hTEAD1, hTEAD2 and hTEAD4) in the others TEADs. The number of cysteine residues that point toward this pocket is also different for each in hTEAD1. C296, C322, C359 and C405 are located quite far from each other, whereas only two cysteine residues were found for hTEAD2 and hTEAD4 (C343 and C380 for TEAD2 and C330 and C367 for TEAD4) and only one cysteine is present in hTEAD3 (C368). At the extremity of the hydrophobic sub-pocket lies a totally non-conserved residue which is small and hydrophilic (C296 for hTEAD1, S305 for hTEAD3 and T304 for hTEAD4) and large and hydrophobic (W308 for hTEAD2).

### III. TEAD targeting

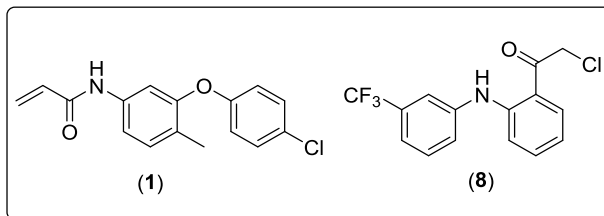
Since 2015, several structures of TEAD with small molecules have been reported and are available at the Protein Data Bank (Table 1 and Figure 4). Either, compounds bind to TEAD at the internal pocket (Table 1, entries 5, 9-15, 22) through covalent bond or not, or at the interface 2 (Table 1, entries 16-20 and 42). Peptides have been specially designed to bind to interface 3 (Table 1, entry 34) or interface 2 (Table 1, entry 43).

hTEAD1	206WQGRSIGTTKLRLVEFSAFLEQQQDPDSYNKHLFVHIGHANHSYSDPLLESVDIRQIYDKF <sup>266</sup>
hTEAD2	218WQARGLGTLARLQLVEFSAFVEPPDAVDSYQRHLFVHTSQHCPSPGAPPLESVDVRQIYDKF <sup>278</sup>
hTEAD3	215WQDRTIASSRLRLLEYSAFMEVQRDPDPTYSKHLFVHTGQTNPAFSDPPIEAVDVRQIYDKF <sup>275</sup>
hTEAD4	214WQGRSVASSKLWMLFSAFLEQQQDPDPTYNKHLFVHTGQSSPSYSDPYIEAVDIRQIYDKF <sup>274</sup>
mTEAD4	207WQGRSIASSKLWMLFSAFLERQQDPDPTYNKHLFVHTSQSSPSYSDPYIETVDIRQIYDKF <sup>267</sup>
hTEAD1	267PEKKGGLKELFGKGPQNAFFLVKFWADLNCNIQ-DDAGA-----FYGVTSQYESSEN <sup>318</sup>
hTEAD2	279PEKKGGLRELYDRGPPHAFFLVKFWADLNWGPSGEEAGAGGSISSGGFYGVSSQYESLEHM <sup>339</sup>
hTEAD3	276PEKKGGLKELYEKGPPNAFFLVKFWADLNSTIQ-EGPGA-----FYGVSSQYSSADSM <sup>327</sup>
hTEAD4	275PEKKGGLKDLFERGSPNAFFLVKFWADLNTNIE-DEGSS-----FYGVSSQYESPEN <sup>326</sup>
mTEAD4	268PEKKGGLKELFERGSPNAFFLVKFWADLNTNID-DEGSA-----FYGVSSQYESPEN <sup>319</sup>
hTEAD1	319TVTCSTKVCSEFGKQVVEKVE <sup>TEYARFENGRFVYRINRSPMCEYMINFIHKLKHLPEKYMMN</sup> <sup>379</sup>
hTEAD2	340TLTCSKVCSEFGKQVVEKVE <sup>TERAQLDGRFVYRLLRSPMCEYLVNFLHKLRLQLPERYMMN</sup> <sup>400</sup>
hTEAD3	328TISVSTKVCSEFGKQVVEKVE <sup>TEYARLENGRFVYRIHRSPMCEYMINFIHKLKHLPEKYMMN</sup> <sup>388</sup>
hTEAD4	327IITCSTKVCSEFGKQVVEKVE <sup>TEYARYENGHYSYRIHRSPMCEYMINFIHKLKHLPEKYMMN</sup> <sup>387</sup>
mTEAD4	320IITCSTKVCSEFGKQVVEKVE <sup>TEYARYENGHYLYRIHRSPMCEYMINFIHKLKHLPEKYMMN</sup> <sup>380</sup>
hTEAD1	380SVLENFTILLVVTNRDTQETLLCMACVFEVSNSEHGAQHHIYRLVKD <sup>426</sup>
hTEAD2	401SVLENFTILQVVTNRDTQELLCTAYVFEVSTSERGAQHHIYRLVRD <sup>447</sup>
hTEAD3	389SVLENFTILQVVTNRDTSQETLLVIAFVFEVSTSEHGAQHVVYKLVKD <sup>435</sup>
hTEAD4	388SVLENFTILQVVTNRDTQETLLCIAVFEVSASEHGAQHHIYRLVKE <sup>434</sup>
mTEAD4	381SVLENFTILQVVTNRDTQETLLCIAVFEVSASEHGAQHVIYRLVKE <sup>427</sup>

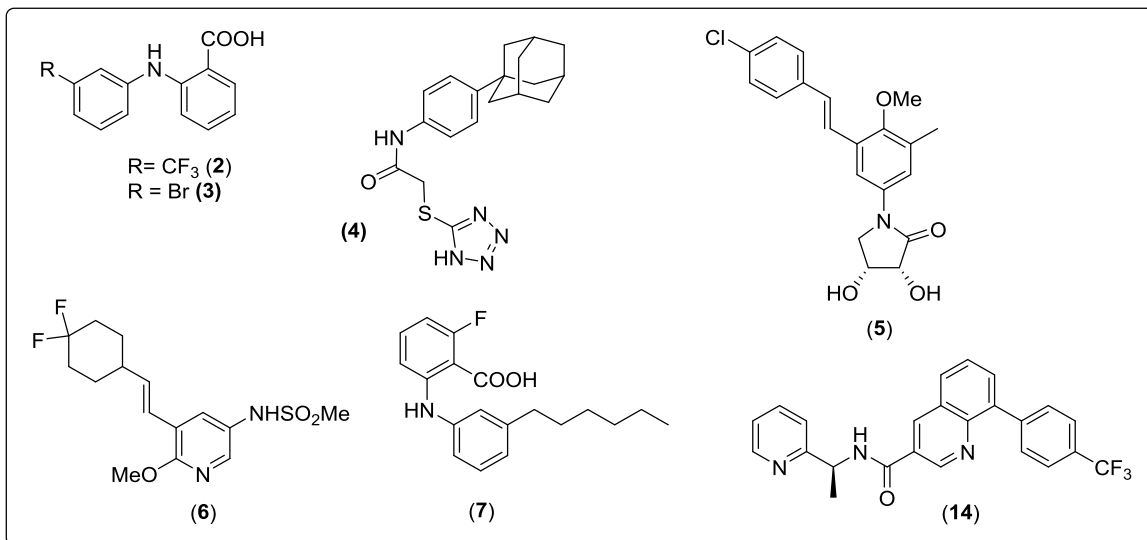
Table 3. Sequence alignments of YBD (YAP-Binding Domain) of crystallized TEADs. Residues of the interfaces 2 (in green) and 3 (in red) are highlighted. Residues of the internal pocket are highlighted in cyan (hydrophobic sub-pocket) and magenta (hydrophilic sub-pocket). Residues making covalent bond with fatty acid are highlighted in bold grey. Not always well-defined loops are in purple box (loop A) and in green box (loop B). Numbering of the different proteins can be found in <http://www.uniprot.org/> (P28347, Q15562, Q99594, Q15561 and Q62296 respectively).

#### A. Targeting TEAD palmitoylation

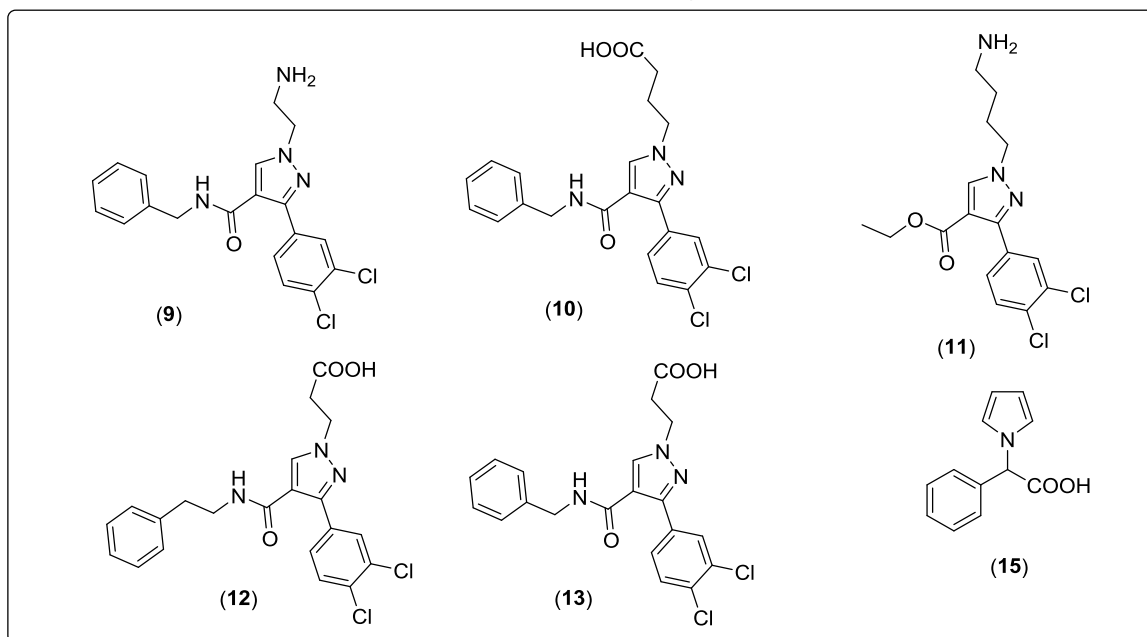
The most druggable site of TEAD is the internal pocket which hosts myristate or palmitate.<sup>51</sup> Many groups have developed ligands targeting this internal pocket. Firstly, Pobbati *et al.* reported niflumic acid and fenamic acid derivatives (Table 1, entries 9-10 and Figure 4, **2-3**)<sup>52</sup> as ligands of this internal pocket, following by W. Xu *et al.* which patented MGH-CP1 and derivatives (Table 1, entry 11 and Figure 4, **4**).<sup>53-54</sup> More recently, Gagnon *et al.* reported the structure of hTEAD2 with LM98, a synthetic analog of niflumic acid<sup>55</sup> (Table 1, entry 14 and Figure 4, **7**) and Holden *et al.* discovered other powerful inhibitors of palmitoylation (Table 1, entries 12-13 and Figure 4, **5-6**).<sup>56</sup> Finally, several groups designed covalent TEAD inhibitors targeting the thiol function of the conserved cysteine residue at the entrance to the internal pocket, bearing chloroacetate groups or an acrylamide moiety (Table 1, entries 5 and 15 Figure 4, **1** and **8**).<sup>57-61</sup> **1** (K-975) was compared to K-886 that is a derivative of K-975 with an acrylamide structure but whose structure is not disclosed in the article.



Internal pocket covalent ligands



Internal pocket non-covalent ligands



Interface 2 non-covalent ligands

*Figure 4. Structures of small molecules that form complexes with TEAD (compound 4 has been redrawn)*

The authors reported that this derivative of K-975 bearing the same reactive function was found inactive without any explanation of this difference. The design of **8** (TED-347) is directly inspired by the structure of **2** (flufenamic acid) and clearly associates the affinity of **2** for the internal pocket and the reactivity of a chloroacetyl group which is known to be one of the highest reactivity in SN2 reactions.<sup>62</sup> Finally, Tang *et al.*<sup>33</sup> reported the crystal structure of VT105 (**14**) with hTEAD3.

#### B. Targeting TEAD-activators protein-protein interaction

Targeting the protein-protein interaction between TEAD and YAP or TAZ has been the earliest strategy leading to the design of YAP-inspired peptides and cyclic peptides<sup>63-64</sup> leading to inhibitors of YAP-TEAD interactions while VGLL4 mimicking led to opposite results: Jiao *et al.*<sup>40</sup> reported YAP antagonists while Adihou *et al.*<sup>41</sup> reported VGLL4 antagonist. In their article, Jiao *et al.* used the mVGLL4 <sup>227</sup>S-<sup>249</sup>I deicosapeptide named TDU2 (Tondu Domain 2) which was optimized into a “superTDU peptide” where TDU2 is linked to YAP’s Ω-loop whereas Adihou *et al.* started from <sup>233</sup>S-<sup>252</sup>A peptide which was modified by a simple D<sup>235</sup>E change in order to optimize an intramolecular ionic bond.

Few small external TEAD ligands were reported. Compound **15** was identified through a fragment based-approach.<sup>65</sup> It binds to YAP-binding interface 2 of TEAD but its affinity for mTEAD4 is very low and it has practically no detectable cellular activity. We recently reported<sup>66</sup> the synthesis of a series of pyrazoles (Table 1, entries 16-20 Figure 4, **9-13**) which also bound to TEAD2 at the interface 2 but at a new cryptic pocket created by the movement of Y382.

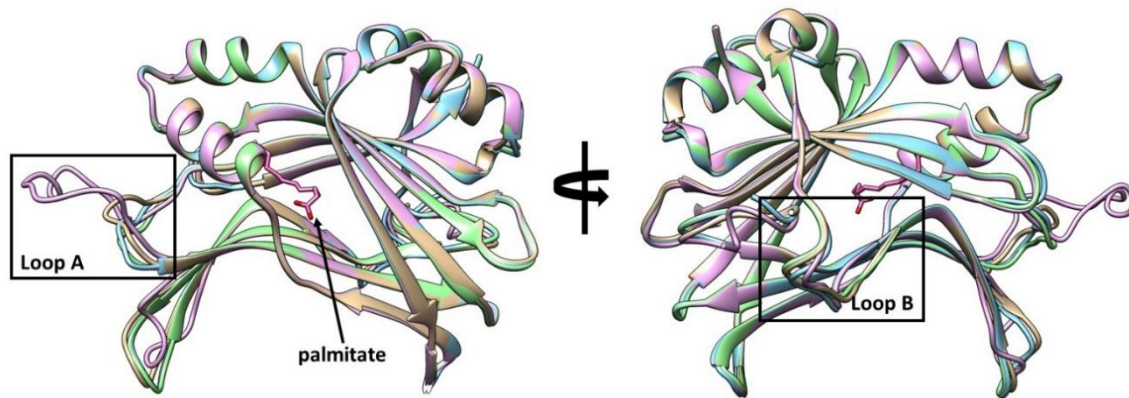
Although initially the most attractive target, no small molecule ligand of interface 3 has been reported to date.

#### IV. Toward specific TEAD targeting

##### A. Through external binding pocket

In the large majority of the available crystal structures of C-terminal domain of TEAD, two mobile loops are not resolved. In all the hTEAD2 crystal structures, a large part of loop A is missing (see purple box, table 3) indicating a high mobility whereas in hTEAD1, 3 and 4 the motif is solved (hTEAD1 PDB code: 6IM5<sup>67</sup>, hTEAD3 PDB code: 5EMW and mTEAD4 PDB code: 5GN0) or misses 3 to 4 residues (for example hTEAD4 PDB code: 5OAQ). This structural difference and the existence of a new pocket close to W308 suggests the possibility to design selective ligands of hTEAD2. The other loop (loop B, see light blue box, table 3) which is not always well-defined in TEAD crystal structure is situated just under

the interface 3. The number of residues is fully conserved whatever the TEAD member but not their nature.

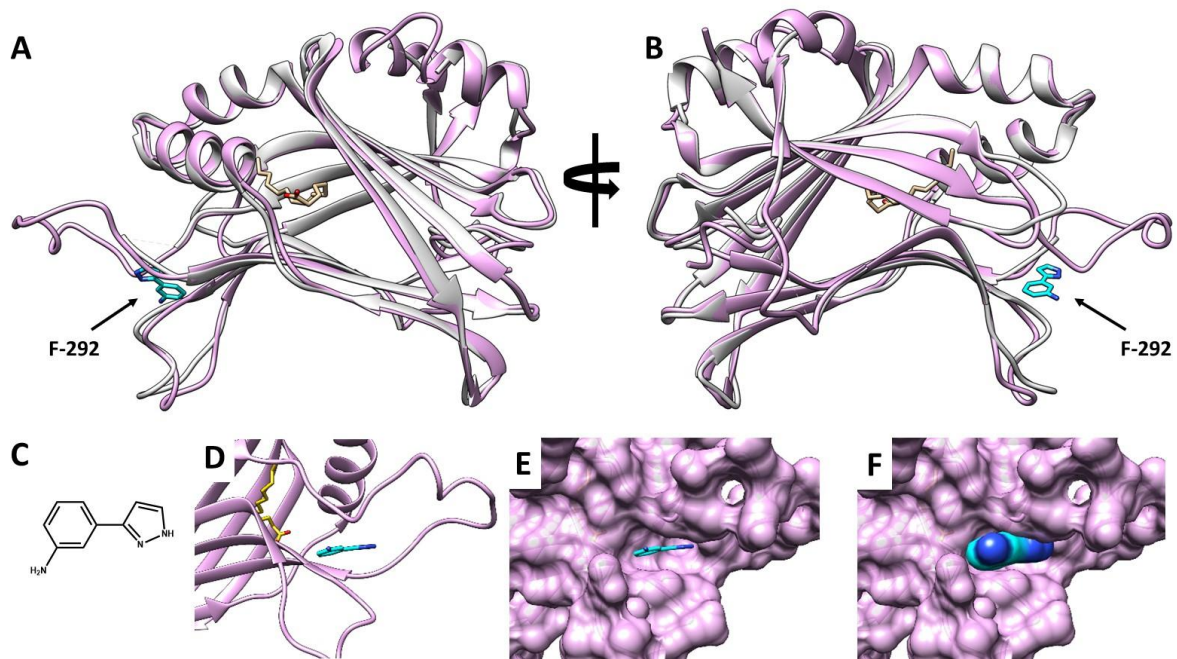


*Figure 5. Superimposition of C-terminal domain of hTEADx (hTEAD1 (tan), hTEAD2 (purple), hTEAD3 (light green) and hTEAD4 (sky blue). Left: view of the palmitate entry, right: opposite view. Loop A corresponds to supernumerary residues of hTEAD2 (see purple box, figure 3) and loop B corresponds to non-conserved residues (see green box, figure 3)*

We anticipate that the basis of the loop A could be an ideal place for a ligand which could be selective for hTEAD2 vs hTEAD1,3,4. Indeed, the pocket present for hTEAD2 is occupied by residues of hTEAD1,3,4. These unsolved loops of C-terminal domain of TEADs are now modeled and available at the AlphaFold2<sup>68</sup> web site (<https://alphafold.ebi.ac.uk/>)(Figure 5).

Concomitantly to this study, we screened a small home-made library of 500 fragments by thermal shift assay (TSA) on the C-terminal domain of hTEAD2 and the 44 fragments with a  $\Delta T_m > 0.7$  °C were soaked with hTEAD2. Amongst the collected crystals, we discovered that one of the fragments (3-(*1H*-pyrazol-5-yl)aniline named F-292)( $\Delta T_m = 0.95$  °C) crystallized in this hTEAD2-specific cavity (PDB code: 7OYJ, supplement table 1). TSA curves of hTEAD2 in the absence or in the presence of F-292 are given in supplementary (Supplement figures 1 and 2). Superimposing hTEAD2 (from AlphaFold2) with the crystallographic structure (Figure 6), shows the fragment F-292 fits very well in the cavity predicted by AlphaFold2. The phenyl ring of F-292 makes hydrophobic interactions with P293 of TEAD2 and the amino group creates hydrogen bond with Q247 whereas at the other end of F-292 the pyrazole ring is close to S323.





*Figure 6. Superimposition of hTEAD2 soaked with F-292 (PDB code: 7OYJ)(grey) and C-terminal domain of hTEAD2 from AlphaFold2 (purple) A: view of the palmitate entry, B: opposite view. Fragment F-292 is in cyan, C: structure of F-292, D, E and F: zoom view of F-292 in the specific TEAD2 pocket*

F-292 occupies a unique pocket specific of hTEAD2. However due to its small size, we expected a low binding affinity towards TEAD and a low efficacy in disrupting the formation of the YAP/TAZ-TEAD protein complex in cells as it was found for fragment **15**.<sup>65</sup> Nevertheless, F-292 constitutes an ideal platform for the design of selective TEAD2 ligands.

## B. Through internal binding pocket

To date, most of the workforce bent toward the internal pocket, and selectivity was mostly reached without being looked at.

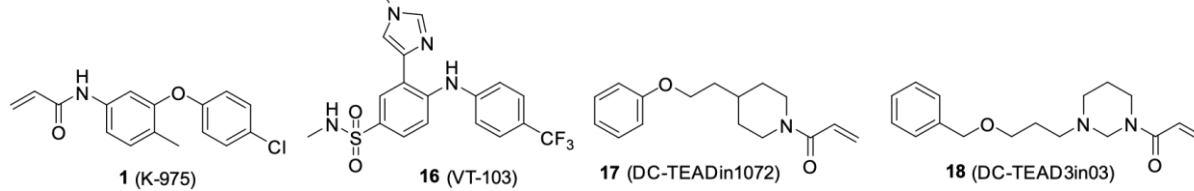
**16** is an optimized analog of a validated hit selected by HTS by Vivace Therapeutics. **16** selectively prevents palmitoylation of TEAD1 in a cell-based palmitoylation assays and inhibits endogenous TEAD1 palmitoylation in cells.<sup>33</sup> TSA experiments showed strong stabilizing effects of **16** on purified recombinant TEAD1 (8.3°C) and in a lesser extent on TEAD2 (4.2°C). YAP/TAZ-TEAD interaction disruption has only been conducted in *NF2 (Neurofibromatosis 2)*-mutant NCI-H2373 cells on TEAD1 and TEAD4 and **16** was found to selectively reduce YAP-TEAD interaction after 4 and 24 h and TAZ-TEAD after 4 h. **16** potently inhibited the proliferation of *NF2*-mutated/deficient cell lines *in vitro*, but failed to show antiproliferation activity in additional mesothelioma cell lines without *NF2* mutations, but harboring alterations in other Hippo pathway core components.

**1** was reported as a novel TEAD inhibitor with anti-tumor effects on pleural mesothelioma.<sup>57</sup> SPR experiments showed that **1** inhibited the interaction of TEAD1 C-terminal binding domain and biotinylated YAP peptide and in NCI-H226 cells, **1** was found to be more active on TEAD1-YAP disruption than on

TEAD4-YAP complex. Although **1** was not claimed to be a selective ligand of TEAD1, it appears it exerted a stronger inhibitory activity towards *NF2*-non-expressing mesothelioma cell lines as found for **16** (Figure 7). **18** was reported to be a selective ligand of TEAD3. It derived from **17** which can inhibit TEAD1/3 at submicromolar level.<sup>69</sup>

These promising strategies share the same drawback: a lack of rational design potential other than the covalent binding. Thus, we focused our interest onto the structural determinants that could and should exist and underlie of the observed selectivity. For this purpose, we performed docking study on these selective ligands (**1**, **16-18**).

Despite that **1**, **17** and **18** are able to covalently bind to TEAD through a nucleophilic attack of thiol function of the conserved cysteine, we did not use covalent docking first intention to avoid to introduce *a priori* bias to the method. Indeed, an effective covalent ligand can be viewed as a specific non-covalent ligand that will undergo covalent bond formation after interaction with its target.<sup>70</sup> Since we were interested in the structural determinant of binding, the use of covalent docking would introduce an *a priori* for the most favorable binding site. In our approach, we looked for binding specificities of the described ligands to observe the emergence of a rational binding pattern. Subsequently, if a covalent



inhibitor specifically binds in a manner that allows reactivity with the thiol function, our model would then validate its specificity.

*Figure 7. Structure of selective ligands of one or two members of TEAD*

Docking of **16** showed that **16** fits well in the internal pocket of TEAD1 and TEAD2 (Figure 8A) with vinascore for the best pose of -7.8 and -8.1 respectively but is inserted into the surface pocket close to loop B of TEAD3 and TEAD4 (Figure 8B) with vinascore for the best pose of -7.6 and -7.3 respectively. Analysis of the residues which surround **16** in the internal pocket of TEAD1 and TEAD2 indicated a critical bend larger in TEAD1 and TEAD2 than in TEAD3 and TEAD4 due to a series of subtle differences (tyrosine in TEAD3 for a phenylalanine in the other TEADs, that was previously identified by Holden and Cunningham<sup>30</sup>, as reported in table 3 (in blue and purple). The N-methylsulfamide warhead is surrounded by several protic residues (serine, cysteine, lysine) that make hydrogen bonds with the oxygen and nitrogen atoms of this moiety (Figures 9).

This docking is in accordance with TSA experiments.<sup>33</sup> In the TEAD palmitoylation assay in cells, HEK293T cells are transfected with myc-TEADx expression plasmid but the transfection seemed to be less efficient with myc-TEAD2 than with the others. It appears **16** seemed to moderately inhibit TEAD2 palmitoylation.

Docking of **1** gave the same results as for **16**. **1** fits in the internal pocket of TEAD1 and TEAD2 (Figure 9A) with score for the best pose of -8.1 and -9.1 respectively but is inserted into the surface pocket close to loop B of TEAD3 and TEAD4 (Figure 9B) with score for the best pose of -6.6 and -6.3 respectively. The discrepancy is more marked in term of scoring for **1** than for **16**.

The best pose of **1** in TEAD1 and TEAD2 showed the vinyl group of **1** is ideally exposed to the nucleophilic attack of C359 or C380. This docking is in accordance with the pull-down experiments in cells<sup>57</sup> showing that **1** inhibits more efficiently YAP/TAZ-TEAD1 interactions than YAP/TAZ-TEAD4 interactions.

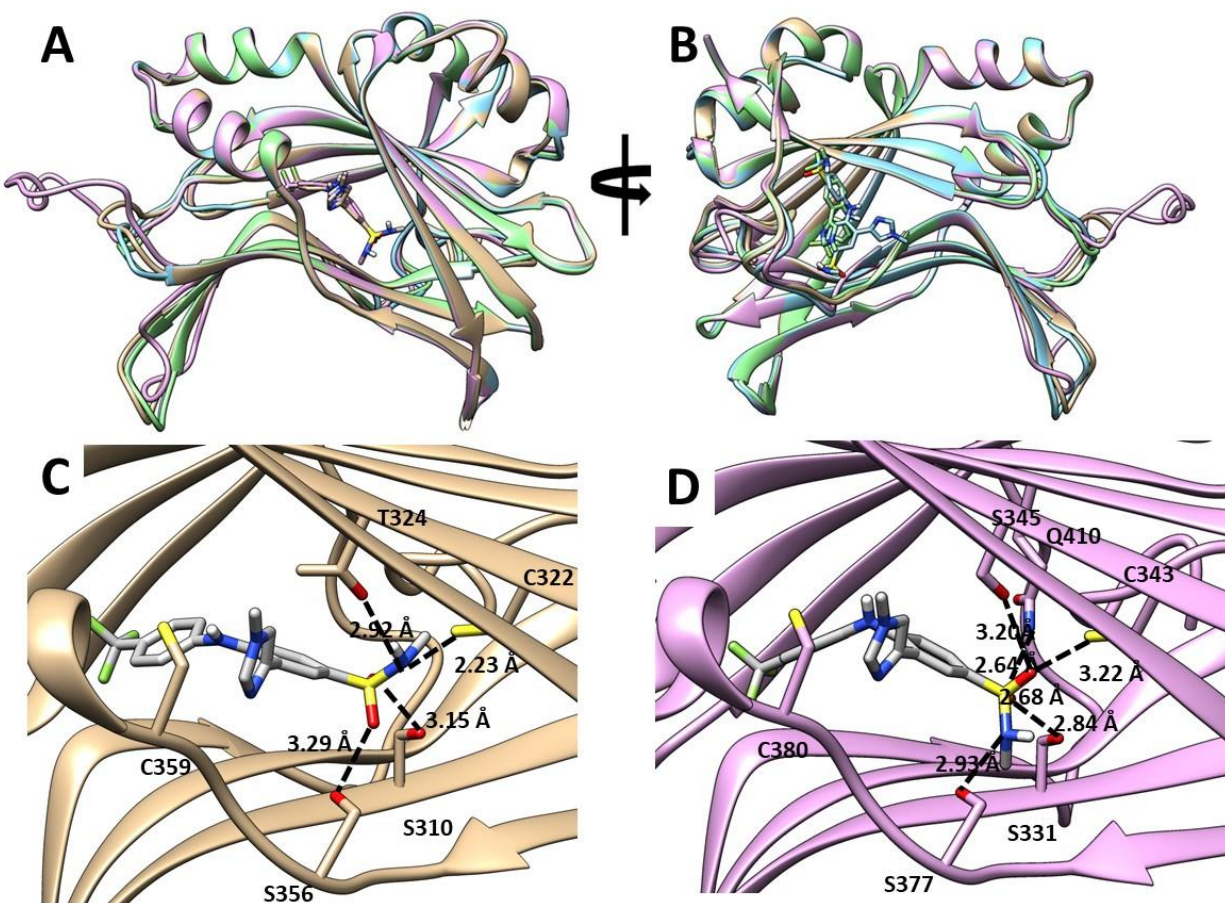


Figure 8. Superimposition of the best poses of **16** docked in C-terminal domain of hTEADx (A view of the palmitate entry: hTEAD1 (tan), hTEAD2 (purple); B opposite view: hTEAD3 (light green) and hTEAD4 (sky blue). Best poses of **16** in C-terminal domain of hTEAD1 (C) and hTEAD2 (D))

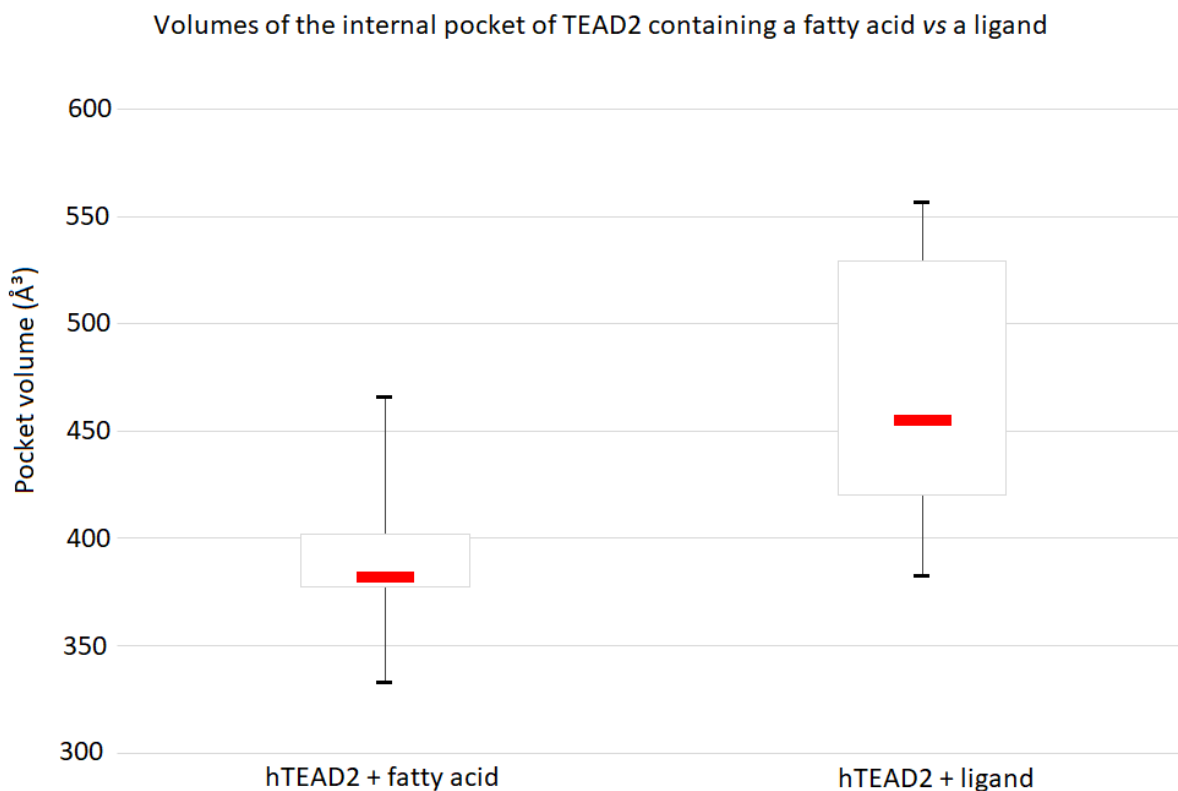
Docking of **17** showed a non-selectivity as **17** fits in the internal pocket of TEAD1, 2 and 4 (best scores: -7.5; -8.1; -7.7 respectively, Supplement figure 3) and in the pocket created by loop B of TEAD3 (best score: -5.4). Docking of **18** showed a non-selectivity as **18** fits in the internal pocket of TEAD1, 2 and 3 (best scores: -7.4; -7.6; -8.1 respectively, supplement figure 4) and at interface 2 of TEAD4 (best

score: -5.8). In TEAD1, 2 and 3, **18** has its vinylsulfamide moiety oriented towards nucleophilic residues (C359 for TEAD1, S331 for TEAD2, K345 for TEAD3). **17** and **18** seemed to be too small and too flexible for the docking to discriminate between the tight and subtle differences of the internal pocket of the four TEAD members. In the original publication<sup>69</sup>, the authors explained the selectivity of **17** for TEAD1 and 3 and of **18** for TEAD3 by docking **17** and **18** using Covalent Docking panel integrated in Maestro 9.2. In their study, they claimed **17** has a steric clash with L383 (TEAD2) and L366 (TEAD4) that are methionine residues in TEAD1 and 3 and **18** could form hydrogen bond with Y230 (TEAD3).

Secondly, we docked covalent ligands **1**, **17** and **18** on C-terminal domain of TEAD1-4 using GOLD.<sup>71</sup> As expected, covalent docking gave solutions for these three acrylamide derivatives for the four C-terminal domains of TEAD and is therefore unable to explain selectivity (Supplement figures 5, 6 and 7).

Finding this potential lack of apparent rational structure-activity relationship, we analyzed the volume of the internal pocket of hTEAD2 for each hTEAD2 monomer using Ligbuilder 3.0 cavity. For crystal structures where the internal pocket is occupied by myristate or palmitate (Table 1, entries 6-20), the volume is  $393.4 \pm 32.7 \text{ \AA}^3$  whereas the volume of soaked hTEAD2 with small molecules is  $468.0 \pm 58.5 \text{ \AA}^3$  (table 1, entries 9-15)(Figure 9). The same difference can be found

in hTEAD1 and hTEAD3. This means that the internal pocket has remarkable plasticity/adaptability to the ligand (fatty acid or exogenous molecule) which makes the design of selective ligands based on steric constraints extremely unlikely.



*Figure 9. Comparison of hTEAD internal pocket volumes. The boxplots represent the median of the values (in red) surrounded by the first and third quartiles (which represent a box around the median) and of the minimum and maximum values (black lines)*



Therefore, we propose here a design of rational selective ligands using our finding. TEAD1 displays two cysteines in its internal pocket that are not conserved on the others, C296 and C405, that could be targeted by covalent binding. Opposingly, TEAD3 lacks two conserved cysteines (V331 and V411) and could therefore be avoided or targeted by more hydrophobic moieties near these regions. TEAD2 noticeably displays a non-conserved tryptophane in the very bottom of its internal pocket, making it smaller and allowing specific targeting through aromatic interactions.

## V. Conclusion

The analysis of the C-terminal domain of the four members of the TEAD family revealed a high conservation of the protein-protein interface with its co-activators but significant differences in regard of the internal pocket : (i) the number of cysteine residues pointing towards the internal pocket could be used to design new ligands specific of TEAD1 (4 cysteines), TEAD2/4 (2 cysteines) or TEAD3 (1 cysteine); (ii) the bottleneck that forces palmitate/myristate to form a bend at C5/C6 (M358/M362 for TEAD1, M379/L383 for TEAD2, M367/M371 for TEAD3 and L359/M363 for TEAD4); (iii) the specific tyrosine residue of TEAD3 (Y230)<sup>30</sup>; (iv) the external loop A of TEAD2 which is close to W308 pointing at the hydrophobic end of the internal pocket.

A lot of effort has been devoted to targeting both the protein-protein interaction of TEADs with its co-activators with moderate success. Most of the effort are now focused on the internal palmitoylated pocket that appears to be able to modulate (mainly inhibit but also activate<sup>72</sup>) the activity of TEADs. Several groups have designed molecules bearing a reactive function to create a covalent bond with the highly conserved cysteine at the entry of palmitate pocket (C380 in hTEAD2). Selective drugs could bear two reactive functions with different reactivity (Michael acceptors, epoxide, sulfoxide, chloroacetamide, nitrile, ketoamide)<sup>73, 74</sup> that could react with two cysteines or with one cysteine and another nucleophile (tyrosine, serine, threonine). To date, all the published strategies are presently lacking a structure-based design as the internal pocket displayed a high plasticity.

Silencing individual members of the TEAD family in hepatoblastoma cell lines gives raise to the overexpression of other members and of certain target genes<sup>75</sup> while in primary human keratinocytes, it is necessary to silent both TEAD1 and TEAD3 to reduce the expression of target genes with a concomitant increase of TEAD2.<sup>76</sup> Therefore, in given cellular conditions, some TEADs can display effects opposing the others, which would lead to a failure of pan-TEAD inhibitors and justify the interest of selective TEAD ligands. Selectivity has already been approached by several groups, and here we propose to design covalent ligands toward non-pan-conserved cysteines. We also demonstrated that a specific pocket

of hTEAD2 can welcome a small molecule (F-292) that could be a new platform for the design of selective ligands of TEAD2.

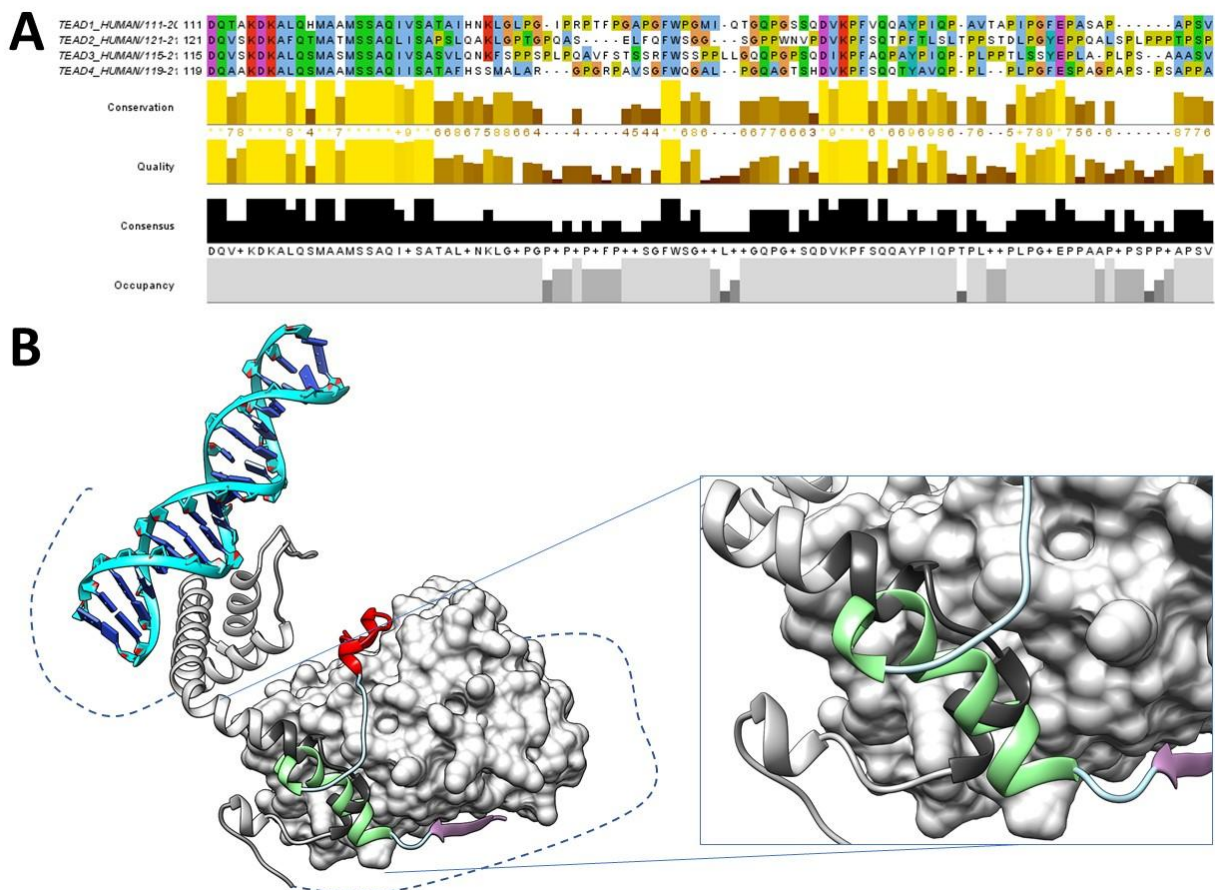
Three molecules (structures are not disclosed) entered in phase 1 clinical trial: VT-3989 (NCT04665206: Evaluation in Patients With Metastatic Solid Tumors Enriched for Tumors With NF2 Gene Mutations) from Vivace Therapeutics in early 2021, followed by IAG933 (NCT04857372: Evaluation in Patients With Advanced Mesothelioma and Other Solid Tumors) from Novartis and finally IK-930 (NCT05228015: Evaluation in Subjects With Advanced Solid Tumors) from Ikena Oncology in the beginning of 2022.

## VI. Perspectives: toward a model of full-TEAD inhibition

In this article, we mainly analyzed the primary and tertiary structures of C-terminal domain of TEADs, their ligands to date and questioned their inhibitor potency. The mechanism of action of the ligands of the internal pocket and the acylation to TEADs activity is still largely unknown, let alone the mechanism of ligands targeting this pocket. Conversely, while the targeting of the protein-protein interaction with the co-activators seems more straightforward, most of the ligands inhibiting the interaction failed to translate to cell activities and cell-active compounds seem unrelated to the binding of co-activators. Such inconsistencies raised a major question about the mechanism of TEADs activation. To date, four

classes of protein partners are known for TEADs: VGLL1-to-3 activate TEAD independently of the Hippo pathway, YAP/TAZ activate TEADs when unphosphorylated (Hippo pathway off), VGLL4 suppresses TEAD by sequestering it onto an abnormal dimer,<sup>77</sup> and finally the FAM181 whose effect toward TEADs are still unknown. It seems that YAP/TAZ binding overwrites the VGLL ones but nothing is known to which extent and why, as their binding determinants are mostly shared (Figure 1).<sup>78</sup> The activation of TEADs leads to the binding of the most N-terminal part to DNA response sequence. But nothing is known about how the binding of an activator to the C-terminal part of TEADs modulate this N-terminal part. The activation of TEADs means that an inactive form should exist, that should be modified by the co-activators. At this point, Alphafold2 provided us with the starting point of a new hypothesis, modeling a part of the linker (between the N-terminal and the C-terminal domains) (Figure 10A) region of TEADs as a bent helix interacting with the interface 2. Regarding the conservation of the sequence, both the N- and C-terminal domain are strongly conserved but the linker region also displays three sub-regions highly conserved, among which one is the one from the bent helix of the Alphafold2 models (Figure 10B in dark grey). Thus, we formulated the hypothesis that TEADs exist in an inactive auto-inhibited form involving the linker region folded around the interface 2 and therefore sequestering the N-terminal domain. This correlated well with the conclusions of

Chène's group on the fact that the binding of the YAP  $\alpha$ -helix to TEAD (interface 2) occurs before any event at the interface 3.<sup>79</sup> This anti-inhibited form involving interface 2 explains both the present observations on the TEADs activation and the relative failure of the PPI inhibitors. Such inhibitors that will out-compete YAP-like binding will, in this hypothesis, also compete with the auto-inhibited form of TEADs, ultimately leading to its activation.<sup>41</sup> This hypothesis, once validated, should lead to a more efficient way to modulate the TEAD activity.



*Figure 10. A: Sequence alignment of the linker between the DNA binding domain and the C-terminal domain of hTEADs; B: Superimposition of hTEAD1 from AlphaFold2 (grey) with the crystal structures of the N-terminal domain of hTEAD1 with DNA (cyan) (PDB code: 5NNX) and The C-terminal domain of hTEAD1 with YAP (interface 1 in purple, interface 2 in green and interface 3 in red) (PDB code: 3KYS). The conserved motif of the linker (in dark grey) interacts on the interface 2 with YAP (and also TAZ or VGLLs) (see zooming). Dotted lines correspond to very low confidences ( $pLDDT < 50$ )*

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### **Author Contributions**

F.T., N.R. and M.L. analyzed TEAD differences and performed docking. M.G. and F.A. produced and purified hTEAD<sub>217-447</sub> protein and performed crystallization and structure determination; J.-F.G. supervised all the X-ray crystallography, TSA study; M.L., J.-F.G., N.R., P.M. and P.C. co-supervised all the project; P.C. supervised writing the article.

## Notes

The authors declare no competing financial interest.

## Biographies

**Maxime Liberelle** holds an MSc in drug design from University of Lille (France), awarded in 2015. He obtained his PhD in structural biochemistry in 2019 on the characterization, structure–function relationships, and therapeutical targeting of oncogenic complex MUC4-ErbB2 under the supervision of Prof. Nicolas Lebègue at the University of Lille in the laboratory of Prof. Patricia Melnyk. After a 2-year postdoc position at the University of Louvain (UCLouvain, Belgium), with Prof. Raphaël Frédérick, he joined the University of Lille, where he is associate professor of biophysics and medicinal chemistry. His research focuses on the protein–protein interaction field, from characterization to targeting.

**Florine Toulotte** obtained her MSc in drug design at the University of Lille. She has just obtained her PhD thesis in medicinal chemistry at the University of Lille in the laboratory of Prof. Patricia Melnyk in dec 2021. Her main research interest concerns the design and synthesis of new small molecules to inhibit protein-protein interaction and their evaluation.

**Nicolas Renault** obtained his PhD degree in molecular modelling at the University of Lille in 2010. He is a research engineer with an expertise in structural



bioinformatics and in silico drug design. Affiliated to the INFINITE research team dedicated to inflammation, he coordinates a platform of protein modelling and virtual screening with successful applications of structure-based design of cannabinergic, purinergic or PPI ligands. He also contributed to many studies focusing onto structure-function relationships of proteins.

**Muriel Gelin** obtained an engineering degree and a PhD, both in chemistry in 1999, worked 6 years in a pharmaceutical company in drug development, and then obtained a Master of Science in structural biology. She works as a CNRS research engineer at the Centre de Biologie Structurale (CBS) in Montpellier. Her fields of expertise are crystallography of biomolecules, synthesis in medicinal chemistry, structural chemoinformatics including virtual screening, molecular modeling, rational drug design (Fragment Based Drug Design), automation by writing programs in python and experimental screening by crystallography and several other techniques such as Thermal Shift Assay or ITC. She is in charge of the ligand screening activity on the CBS crystallographic platform.

**Frédéric Allemand** is an engineer from Toulouse National Institut of Applied Sciences. He obtained his PhD in biochemistry and molecular biology at the university of Paris-Jussieu in 2006. He is Research Engineer in the Scientific Research National Center at the Montpellier Structural Biology Center (CBS). He studied for several years the regulation of ribosomal genes expression in

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**Patricia Melnyk** Patricia Melnyk earned her degree in Chemical Engineering from Chimie ParisTech (1989) and a PhD in Organic Chemistry from the Institut de Chimie des Substances Naturelles (Paris 6 University, 1993). She is Professor of Chemistry at the Faculty of Pharmacy of Lille University and the team leader of “Brain Biology & Chemistry” team in the Lille Neuroscience & Cognition research center (Inserm UMR-S1172). Her major research interests focus on medicinal chemistry and chemical biology in the area of neurodegenerative diseases and cancer. She cofounded AlzProtect, a start-up dedicated to the clinical development of drug candidates for Alzheimer and neurodegenerative diseases. She is at the origin of the discovery of AZP2006, a drug candidate in clinical phase II.

**Jean-François Guichou** obtained his PhD in Organic chemistry at the University of Lausanne in 2002. He is professor of structural biology and drug design at the University of Montpellier, and his expertise is in medicinal chemistry, FBDD, drug design and X-ray crystallography. He worked in the field of antiviral agents (inhibitors of cyclophilin), infectious diseases and cancer. He is the scientific director of AGVDiscovery, a company developing targeted therapies in oncology.

**Philippe Cotelle** obtained his PhD in Spectrochemistry at the University of Lille in 1987. He is professor of organic chemistry at the National Graduate School of Engineering Chemistry of Lille – Centrale Lille Institut, and his expertise is in medicinal chemistry and translational research. He worked for several years in the field of antiviral agents (HIV-1 integrase, HIV-1 and HBV ribonuclease H and other two-magnesium viral enzymes) and has coordinated several national projects in viral diseases. He moved on the area of protein-protein interactions and is interested in developing new drugs/chemical tools targeting the final effectors of the Hippo pathway.

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## **ACCESSION NUMBERS**

The atomic coordinates and structure factors for hTEAD2-**F292** has been deposited at the PDB with the code PDB: 7OYJ. Authors will release the atomic coordinates and experimental data upon article publication.

## **ABBREVIATIONS**

Birc-5      Baculoviral Inhibitor of Apoptosis Repeat-Containing 5

CTGF      Connective Tissue Growth Factor

Cyr61      Cysteine-rich angiogenic protein 61

GOLD      Genetic Optimization for Ligand Docking

HEK      Human embryonic kidney

NF2      Neurofibromatosis 2

NMR      Nuclear Magnetic Resonance

OCT4      Octamer-binding transcription factor 4

PDB      Protein Data Bank

Sox2      SRY (sex determining region Y)-box 2

SPR      Surface Plasmon Resonance

TAZ      Transcriptional coactivator with PDZ binding motif

TBX5      T-box transcription factor 5

TDU	Tondu domain
TEAD	Transcriptional Enhanced Associate Domain
TSA	Thermal Shift Assay
VGLL	Vestigial Like Protein
WWTR1	WW domain-containing transcription regulator protein 1
YAP	Yes Associated Protein
YBD	YAP Binding Domain

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