https://doi.org/10.17952/38EPS.2026.P476

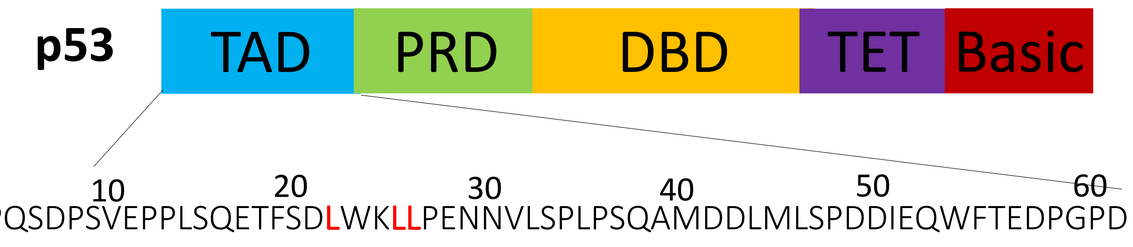
Conformational complexity of the disordered N-terminal region in biological function of tumor suppressor p53 protein

Elena Zindovic, Mo'ath Yousef, Edwige Voisset*, Vladimir Torbeev*

Laboratory of Biosystems Chemistry, École supérieure de biotechnologie de Strasbourg, UMR7242, CNRS and Université de Strasbourg

P53 and TAD

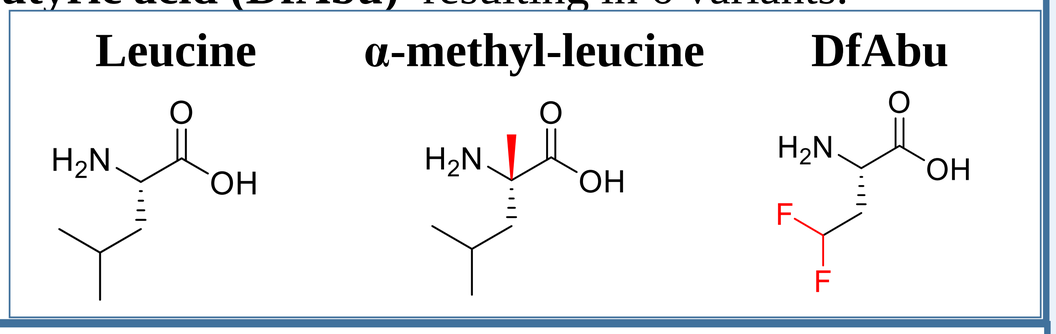
p53 is a **transcriptional activator**, involved in a majority of cancers. It's sequence contains several domains **Transactivation domain (TAD) is an intrinsically disordered region** and binds with various proteins like **CREB-binding protein** (CBP/p300), Double minute 2 protein (HDM2) and many others.[1]
To modify its interactions, TAD variants were synthesized with α-methylated leucine and fluorinated amino acids.



6 variants of TAD with modified LXXLL motif (residues 22-26)

The **LXXLL motif of TAD** was targeted because it is known to **mediate many protein-protein interactions**. This conserved modification sequence mediates **particular interactions with nuclear receptors and their coactivators**, making it a reliable choice for motif to target.[2] Here, only one leucine residue of the LXXLL motif was modified per variant, being individually replaced either by an **α-methylated leucine or by 4,4-difluoro-2-aminobutyric acid (DfAbu)** resulting in 6 variants.

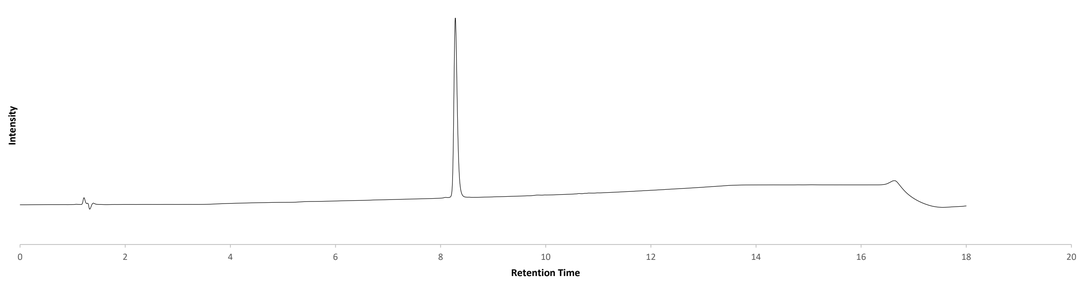
We used **α-methylated leucine** to conformationally **constrain the backbone** and **stabilize helical conformations**.[3] **DfAbu** was chosen as a nearly **isosteric fluorinated analogue** of leucine, while its fluorine atoms also make it as a valuable ^{19}F NMR probe.[4]

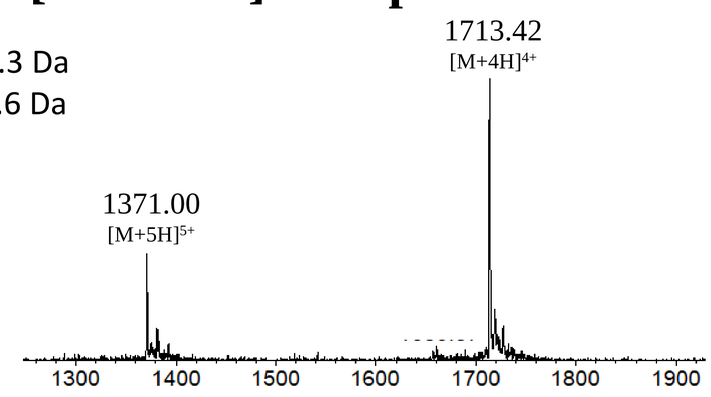


HPLC and mass-spectrometry analysis of the [DfAbu25]TAD-p53 variant

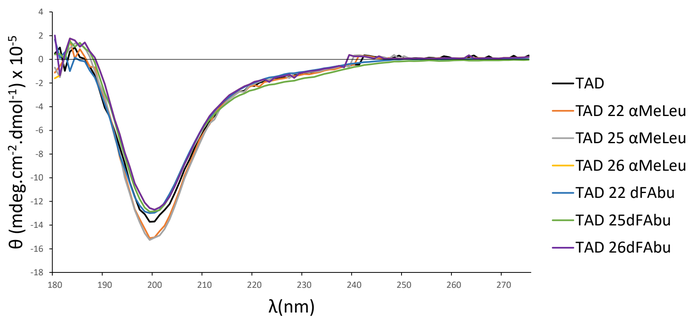
TAD-1-61NH2-25DfAbu
Nle-EEPQSDPSVEPPLSQETFSDLWKL(dFAbu)LPENNVLSPLPSQA-Nle-DDL-Nle-LSPDDIEQWFTEDPGPD-NH2

Mass calculated : 6852.3 Da
Mass measured : 6849.6 Da

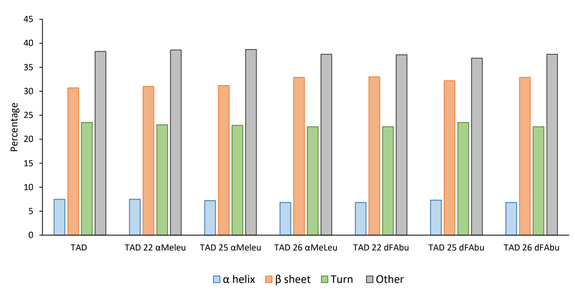




Circular Dichroism of TAD and its variants alone and in complex with NCBD

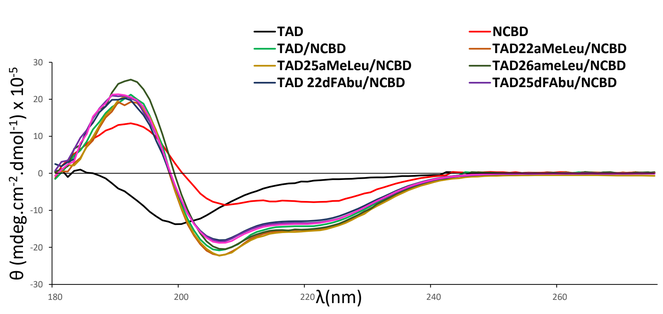


(CD) spectra of TAD and variants in 25 mM phosphate buffer at pH 7 at 25 °C (concentration of protein = 25µM)

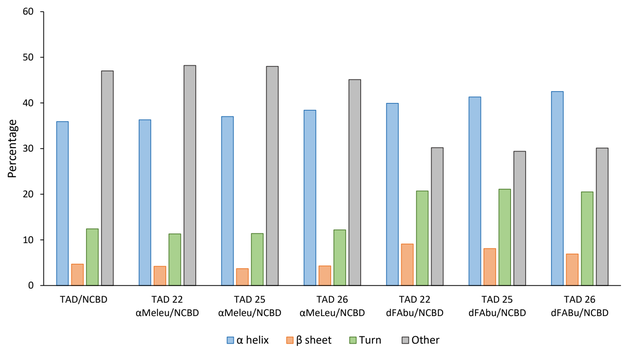


Graphic representation of percentage of α helix, β sheets, turns and others for each variants of TAD estimated using CDPro software (CONTINILL method)

TAD analogs do not alter the overall secondary structure of the transactivation domain, preserving its native conformation.



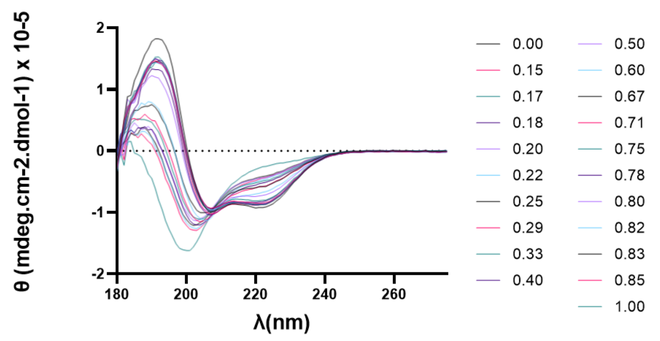
(CD) spectra of TAD and its α methyleucine variant, NCBD and their 1:1 complex in 25 mM phosphate buffer at pH 7 at 25 °C (concentration of protein = 25µM)



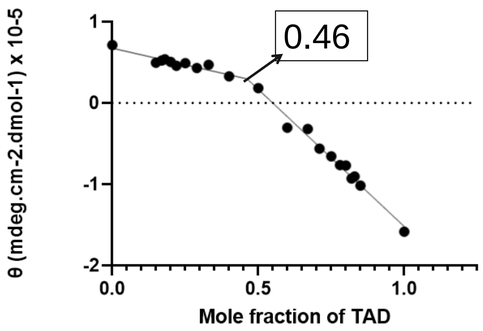
Graphic representation of percentage of α helix, β sheets, turns and others for each variants of TAD, NCBD and their 1:1 complexe estimated using CDPro software (CONTINILL method)

TAD and α-methylated analogs exhibit similar proportions of α-helices, β-sheets, turns, and other secondary structures. In contrast**, the fluorinated analog shows an increase in turns** and a decrease in other structures.

Stoichiometric Analysis of the TAD–NCBD Complex by Job Plot



Graphic representation of TAD–NCBD binding at different concentrations in 25 mM phosphate buffer, pH 7, 25 °C (protein concentration = 25 µM), proportions of TAD are indicated



Since the plot exhibits a maximum at a **TAD mole fraction of 0.5, it** indicates the formation of a **1:1 complex between TAD and NCBD.**

Graphic representation at **198 nm** of TAD–NCBD binding at different concentrations with proportions of TAD indicated

Conclusions:

- **Six new TAD-p53 variants** were **synthesized** incorporating either **α-methylated leucine or DfAbu substitutions.**
- CD spectra indicate that **TAD and its analogues adopt similar secondary structures** in the free state. Upon binding to NCBD, α-methylated variants display behavior comparable to the wild type, whereas **fluorinated analogues show altered secondary structure** profiles. These results suggest that **fluorination affects the folding-upon-binding mechanism**, while **α-methylation largely preserves** it.
- The Job plot exhibits a maximum at a TAD mole fraction of 0.5, confirming the **formation of a 1:1 complex between TAD and NCBD.**

References:

[1] Raj, N.; Attardi, L. D. The Transactivation Domains of the P53 Protein. *Cold Spring Harb. Perspect. Med.* **2017**, *7* (1), a026047.
[2] Plevin, Michael J., Morgon M. Mills, et Mitsuhiko Ikura. « The LxxLL motif: a multifunctional binding sequence in transcriptional regulation ». *Trends in Biochemical Sciences* 30, nº 2 (2005): 66-69.
[3] M. Crisma and C. Toniolo , *Biopolymers*, 2014, **104** , 46
[4] Marsh, E. Neil G., et Yuta Suzuki. « Using 19F NMR to Probe Biological Interactions of Proteins and Peptides ». ACS Chemical Biology 9, no 6 (2014): 1242-50.