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Protein-Protein Interactions: Unraveling the link between HTLV-1 Tax-1 oncoprotein and human *Drosophila discs* large tumor suppressor

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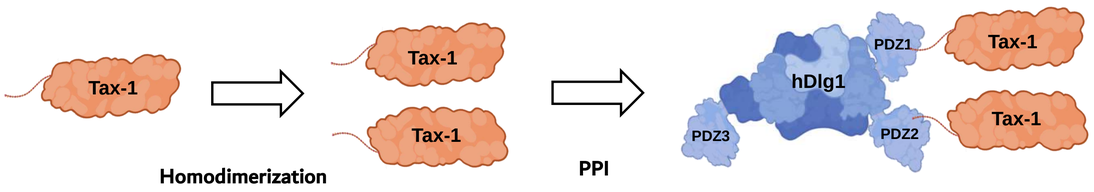
INTRODUCTION [I]

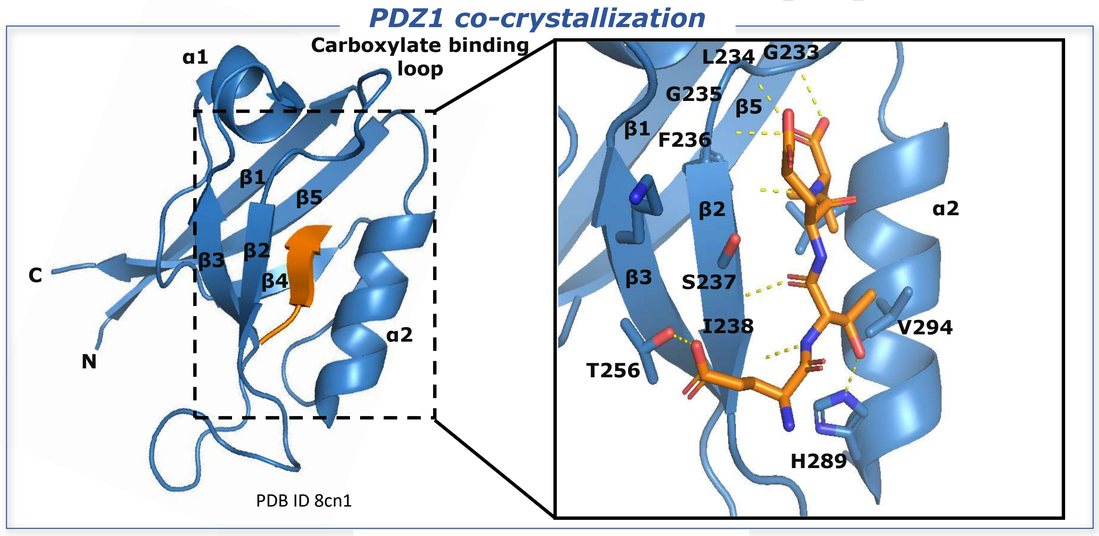
Tax-1 is the most pathogenic protein of the **human T-cell leukemia virus type 1 (HTLV-1)**, an oncogenic virus responsible for the onset of an aggressive form of cancer, **adult T-cell leukemia (ATL)**. The oncoprotein undergoes **protein-protein interactions (PPI)** with a plethora of host proteins, such as the human homologue of the ***Drosophila discs large*** **(hDlg1) tumor suppressor** which is implicated in HTLV-1's oncogenic abilities.[1]This study aimed at the characterization of the interaction between **HTLV-1 Tax-1** and **hDlg1 PDZ domains**.[2]



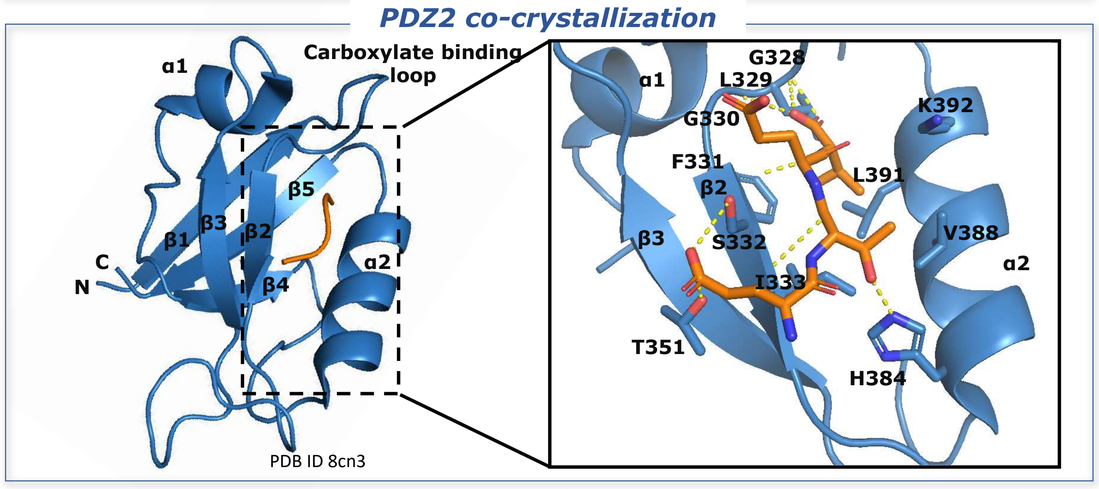
The interaction was characterized through **nuclear magnetic resonance** (**NMR**) and **X-ray crystallography**, additionally **isothermal titration calorimetry** (**ITC**) was utilized to quantify the strength of the respective interactions. Our work provides structural insights essential in the pursuit of **PPI inhibitors**.

X-RAY CRYSTALLOGRAPHY [III]

PDZ1 co-crystallization

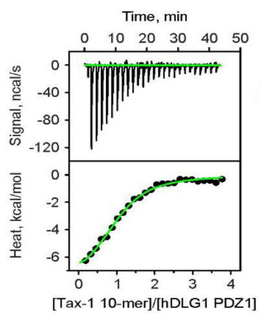


PDZ2 co-crystallization



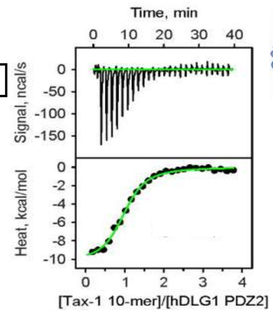
- Residues experiencing the largest spectral changes in NMR (**G235**, **F236** and **S237** for PDZ1 & **G330**, **F331** and **S332** for PDZ2) are accounted for in the crystal structure.
- Binding observed in the crystal structure is **compatible** with the measured spectral changes by solution NMR.

ISOTHERMAL TITRATION CALORIMETRY [IV]



PDZ1 + **Tax-1 10-mer**

- K_D= **2.7** ± 0.3 µM
- ΔH = **-8.0** ± 0.3 kcal/mol
- n = **1.03** ± 0.03



PDZ2 + **Tax-1 10-mer**

- K_D= **0.9** ± 0.1 µM
- ΔH = **-10.4** ± 0.2 kcal/mol
- n = **0.99** ± 0.02

- Both interactions are **enthalpically** driven, with the decamer binding stronger to **PDZ2**.

CONCLUSIONS [V]

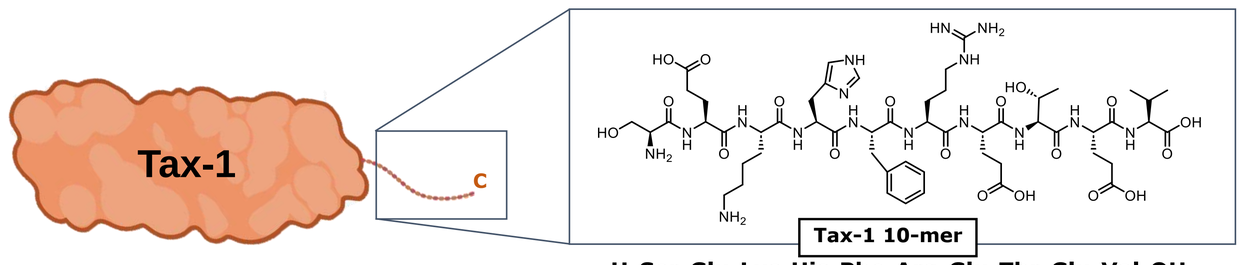
- Solution NMR revealed the binding hotspots to be located in the **β1/β2 loop**, **β2 strand** and **α2 helix.**
- Only the last four amino acids (**H-Glu-Thr-Glu-Val-OH**) of the Tax-1 10-mer seem to be essential for the binding.
- Tetramer bound PDZ domains crystals revealed a binding modality congruent with the hotspots observed by NMR.

PERSPECTIVES [VI]

- Structural insights gained by X-ray crystallography and NMR could be utilized to find potential PPI inhibitors (**small molecules and peptides**).

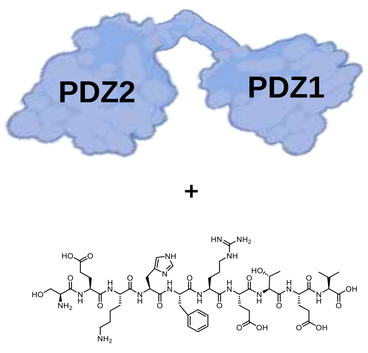
NUCLEAR MAGNETIC RESONANCE [II]

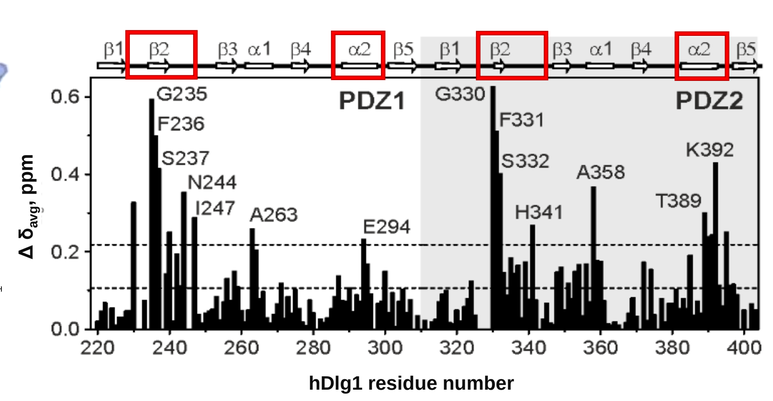
PDZ domains predominantly interact with the *C*-terminal tail of their binding partners, appropriately called **PDZ-binding motif (PBM)**,[3] thus a similar interaction was expected with Tax-1. Peptides mimicking the *C*-terminal tail of the Tax-1 protein were synthetized and their respective interactions with the PDZ domains was evaluated through **[^{1}H,^{15}N] heteronuclear single-quantum correlation (HSQC)**.



H-Ser-Glu-Lys-His-Phe-Arg-Glu-Thr-Glu-Val-OH

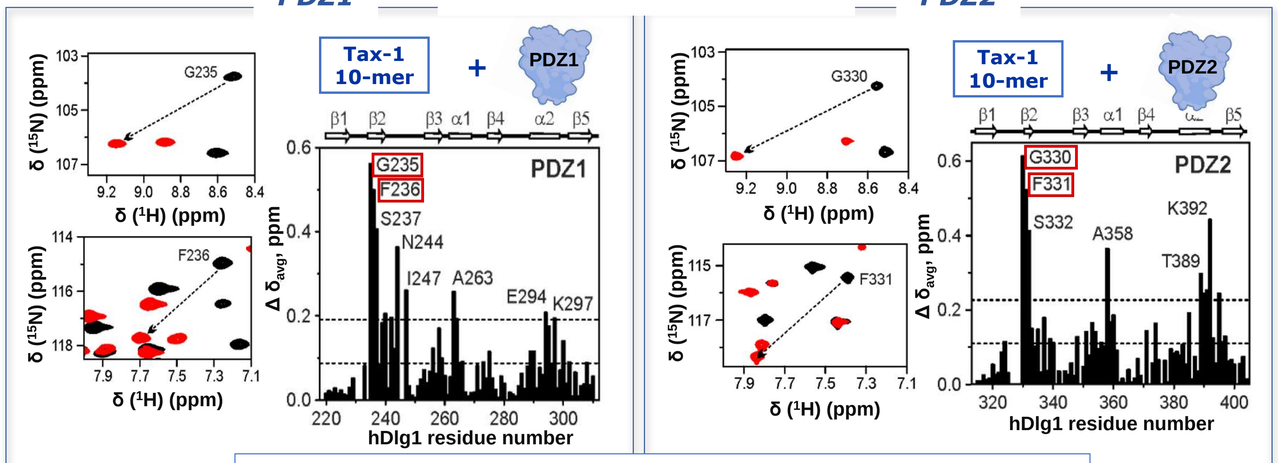
Tandem PDZ1+2





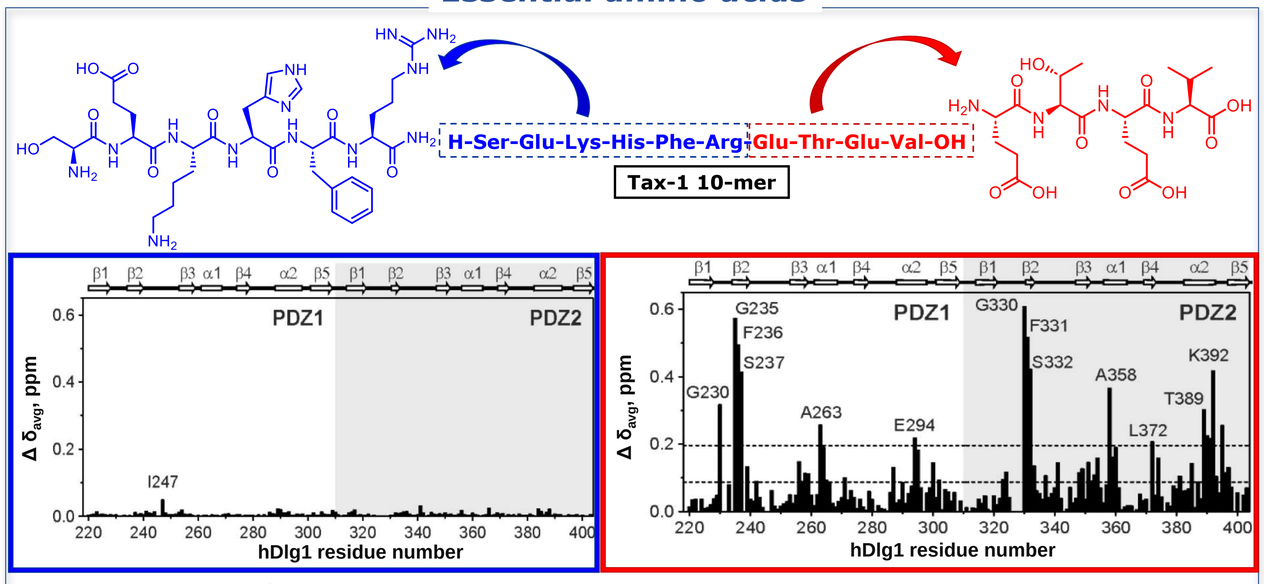
- Significant spectral changes for residues located in **β1/β2 loop**, **β2 strand** and **α2 helix**

Individual domains



- Highly similar binding modalities between the tandem and the individual domains.
- Residues G235, F236, G330 and F331 experience the largest **spectral changes**.

Essential amino acids



- When split in two parts, only the last four amino acids cause spectral changes

H-Glu-Thr-Glu-Val-OH is the essential part for the interaction.

ACKNOWLEDGEMENTS

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