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# Unraveling the binding mechanism of macrocycle peptides to PD-L1 through computational approaches

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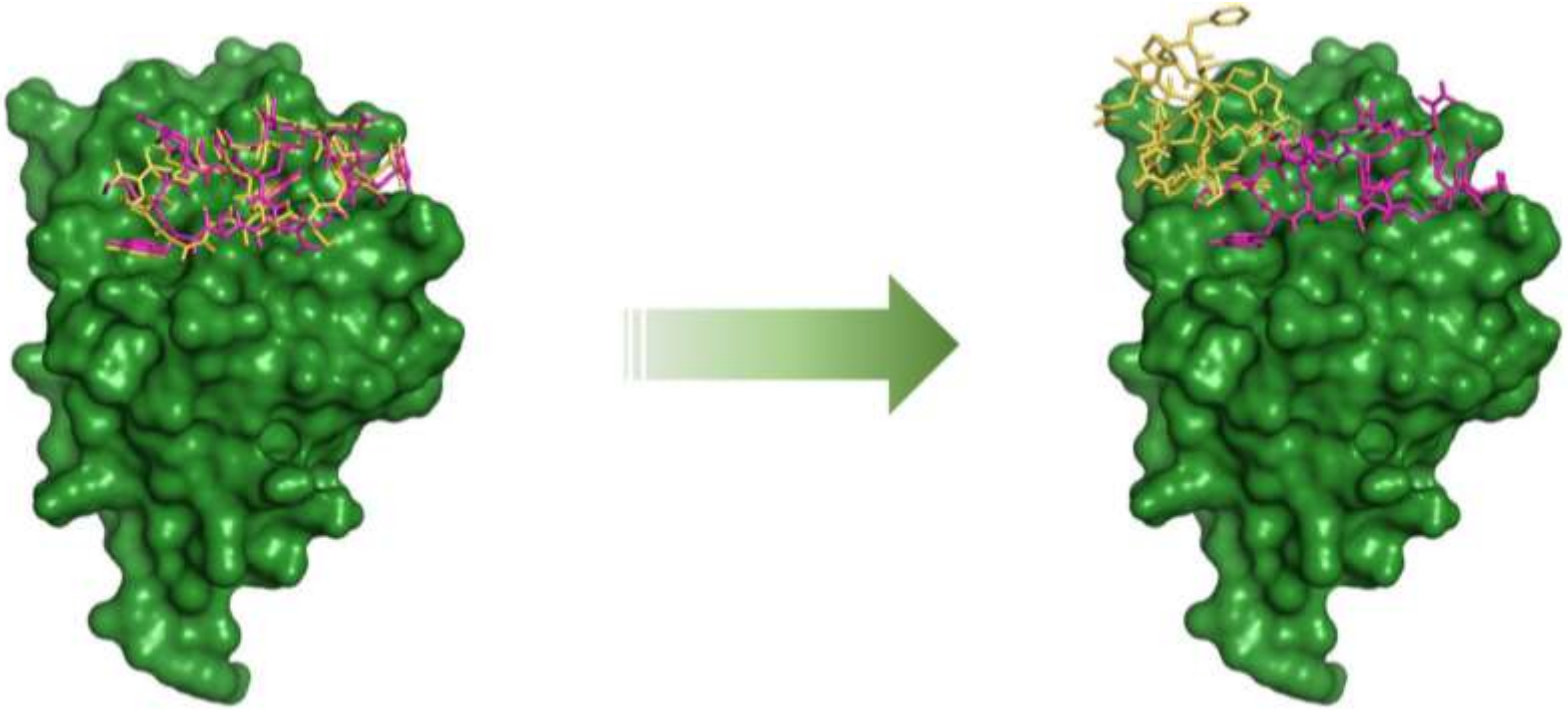
# Unraveling the binding mechanism of macrocycle peptides to PD-L1 through computational approaches

**Graphical Abstract**



## Abstract

PD-1, and its ligand PD-L1, represent a well-known immune checkpoint involved in the silencing of T-cells in the tumor environment. For this reason, they are the target of several mAb that are clinically used for cancer treatment with extraordinary results in some cases. Small molecule inhibitors of PD-L1 are under investigation as well, but they have been demonstrated to cause the dimerization of PD-L1. In the present work, we focused on peptide macrocycles that combine the specificity of mAb with smaller dimensions, better bioavailability, and lower production costs.

In the attempt to understand the leading mechanism driving the binding of the known macrocycles to PD-L1, we focused on co-crystallized macrocycles (PDB IDs: 6PV9 and 5O4Y). These two ligands differ for just one residue (serine and sarcosine) but this difference accounts for an activity gap of two orders of magnitude (pIC50 8.79 and 6.24, respectively).

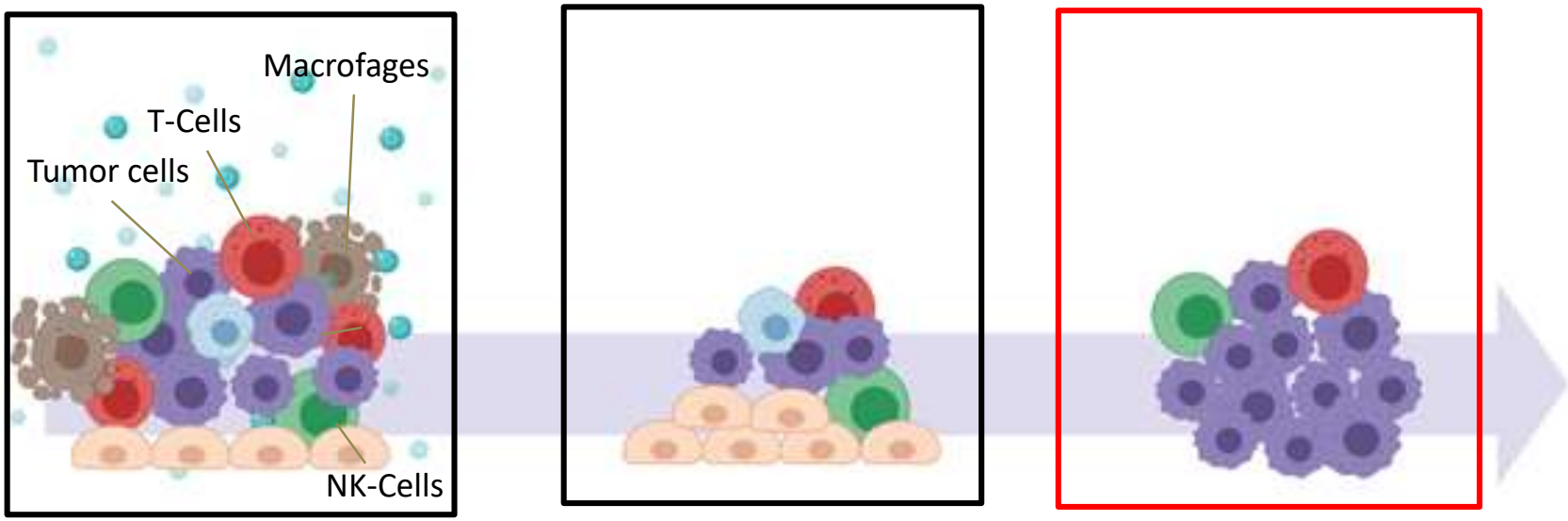
As the analysis of crystallographic binding geometry does not provide explanations, we carried out a 500 ns molecular dynamics simulation on both complexes and the PD-L1 apo-form, aimed to get more insight into the binding process.

The MD simulation revealed a different behavior of the two peptides: the most active resulted stable while the less active detaches from the target macromolecule maintaining a hydrophobic interaction with PD-L1 Tyr123. Interestingly, the same site was also detected by the analysis carried out with TRAPP (TRAnsient Pockets in Proteins), indicating it as a relevant hot spot to be exploited in the PD-L1 ligand design.

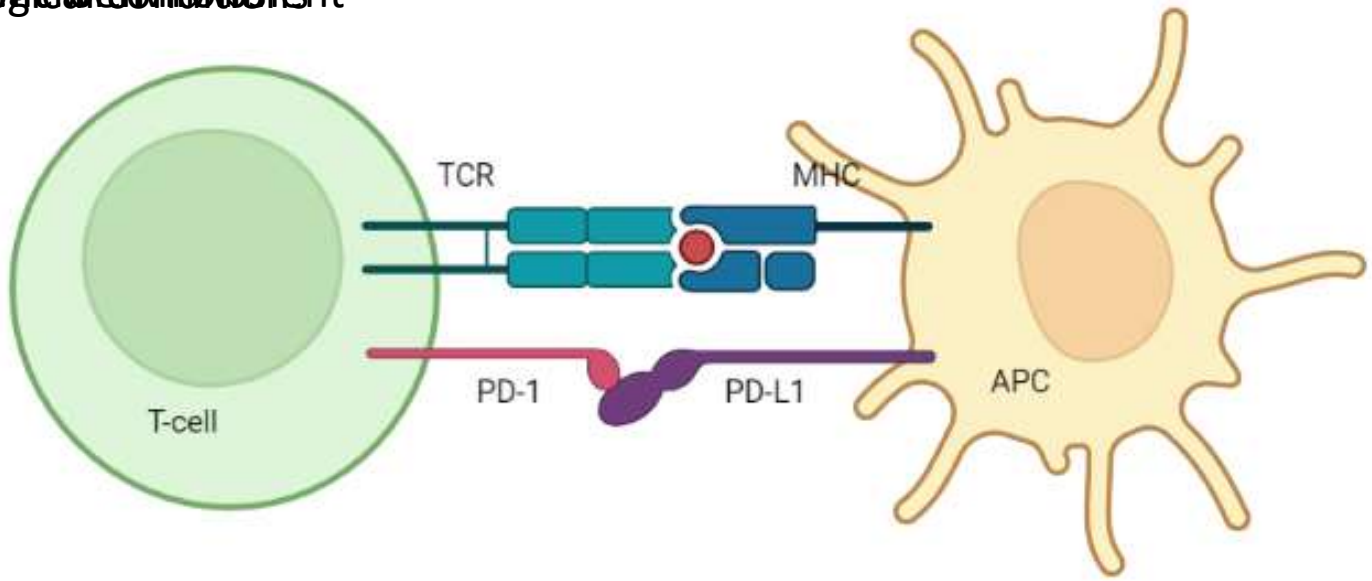
**Keywords:** Cancer Immunotherapy; Immune checkpoint; PD-L1; molecular dynamics

# Cancer Immunotherapy

Cancer cells generate an immunosuppressive environment (TME, tumor microenvironment) where they can proliferate.



# Immune checkpoint

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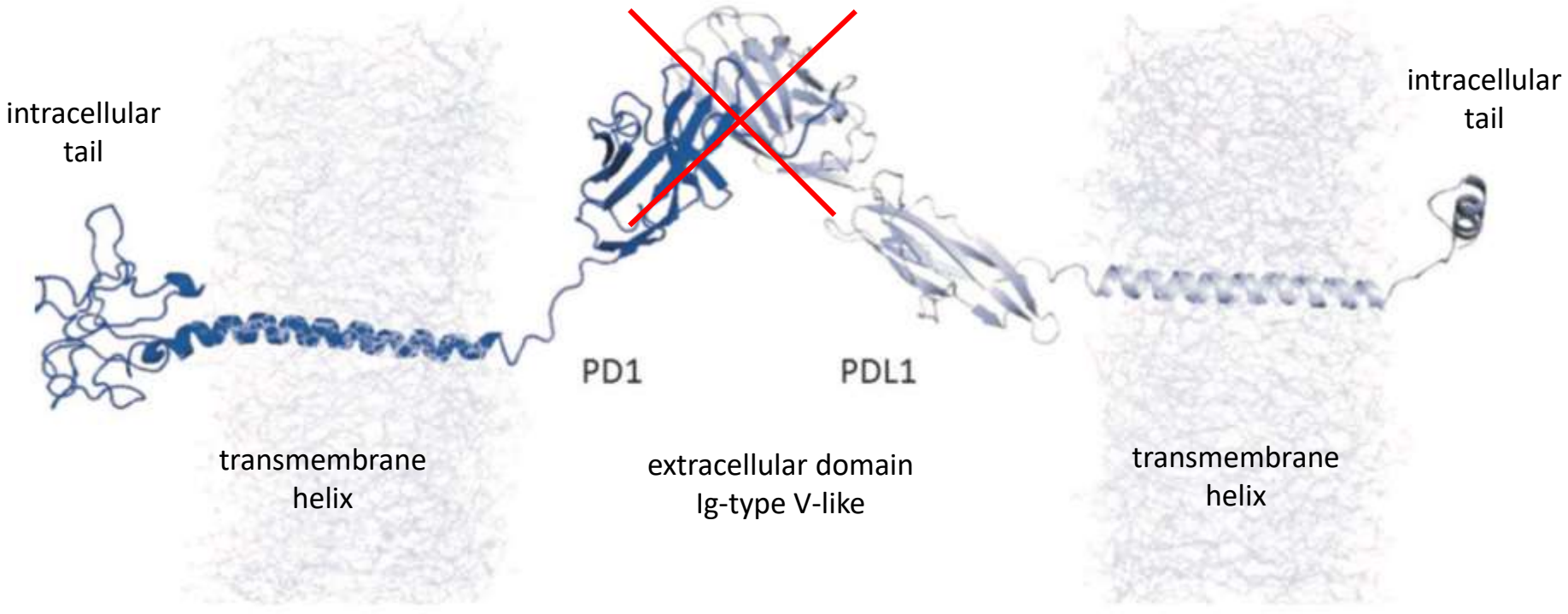
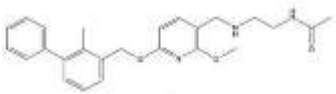
# PD-1 and PD-L1 binding and inhibitors



**Small molecules** → PD-L1 dimerization

**Macrocyclic peptides**

# Focusing on macrocyclic peptides (MPs)

- mAbs specificity
- lower MW
- better bioavailability
- more resistant to hydrolysis than linear peptides
- no PD-L1 dimerization

## Our aim

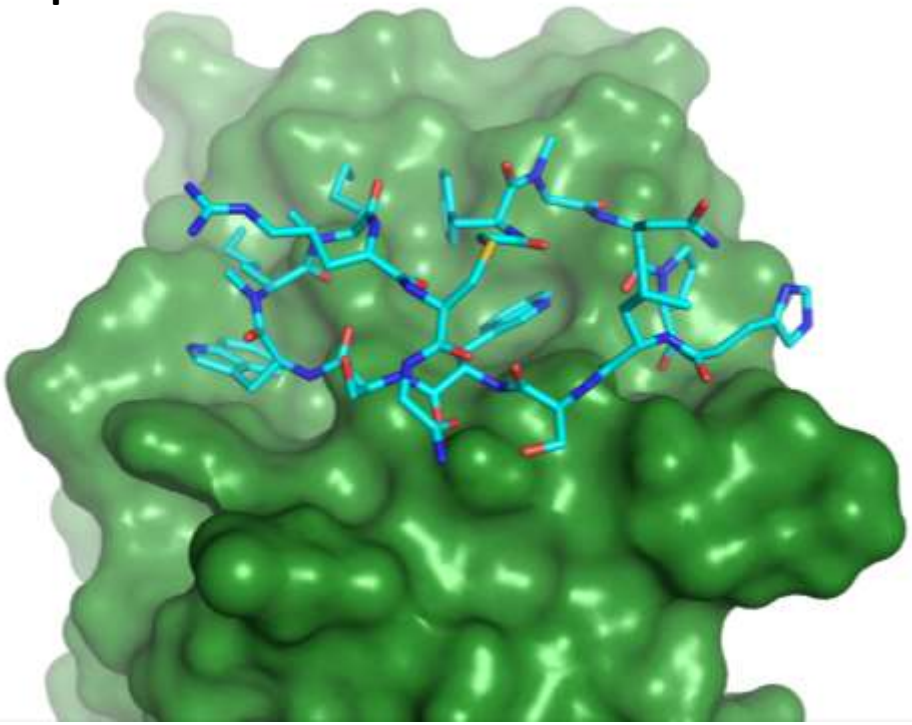
To get better insight on the MP binding to PD-L1 to design smaller, potent and selective ligands.

# Available structural data

**5O4Y**

Resolution: 2.3 Å

**pIC$_{50}$: 6.24**

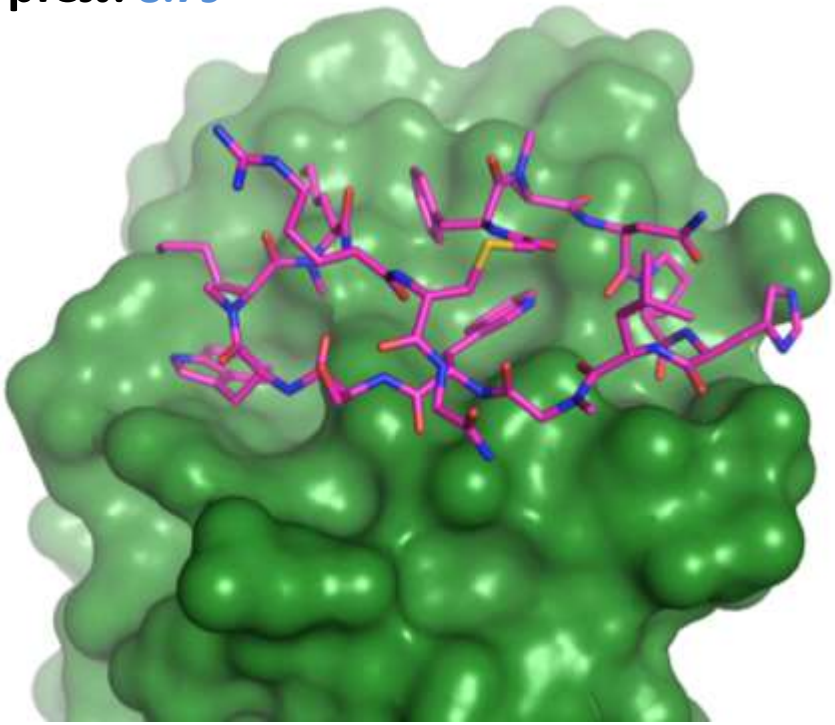


Magiera-Mularz, Angew. Chemie, 2017, 56, 13732

**6PV9**

Resolution: 2.0 Å

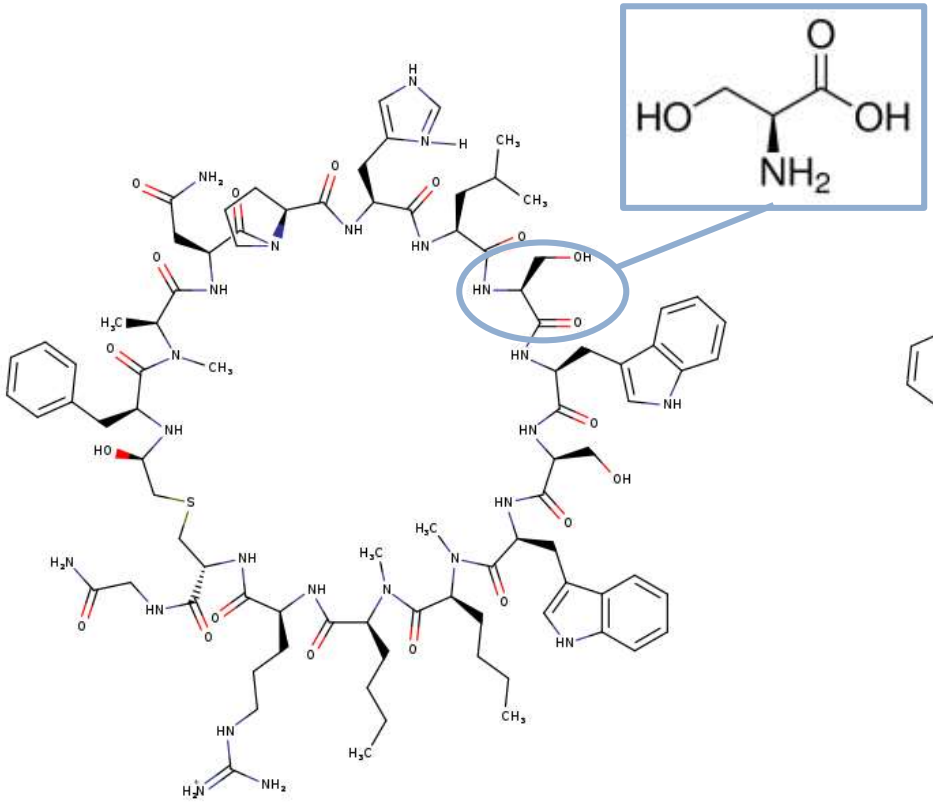
**pIC$_{50}$: 8.79**



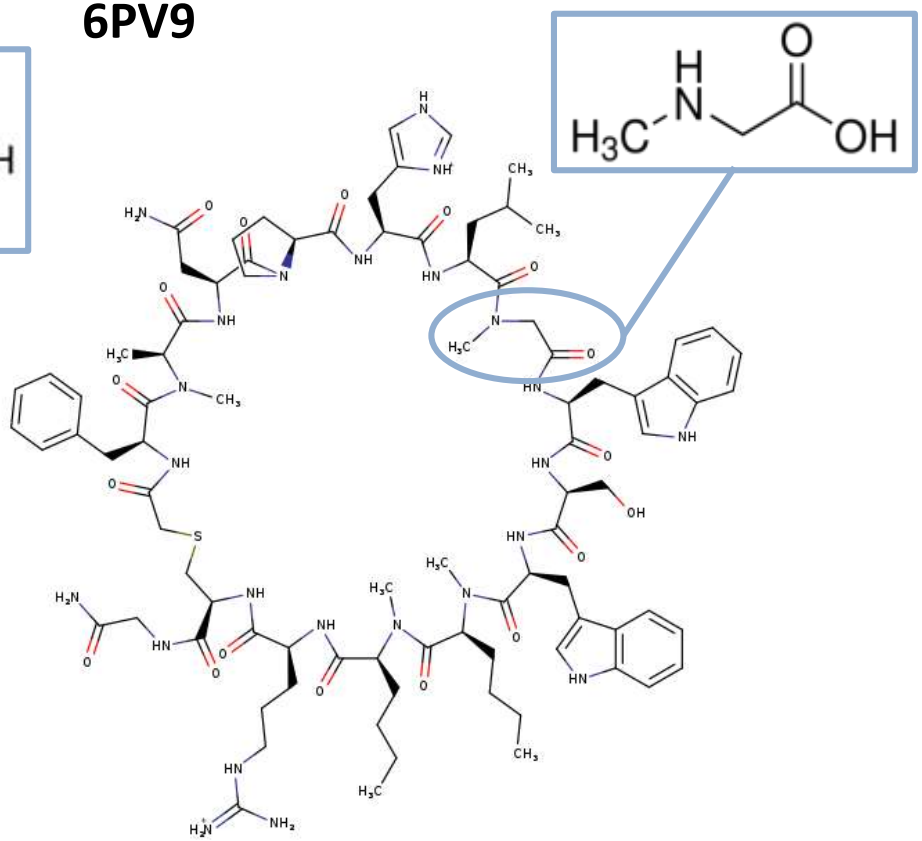
Niu, Biochemistry, 2020, 59, 541

# Macrocycle structure

**5O4Y**

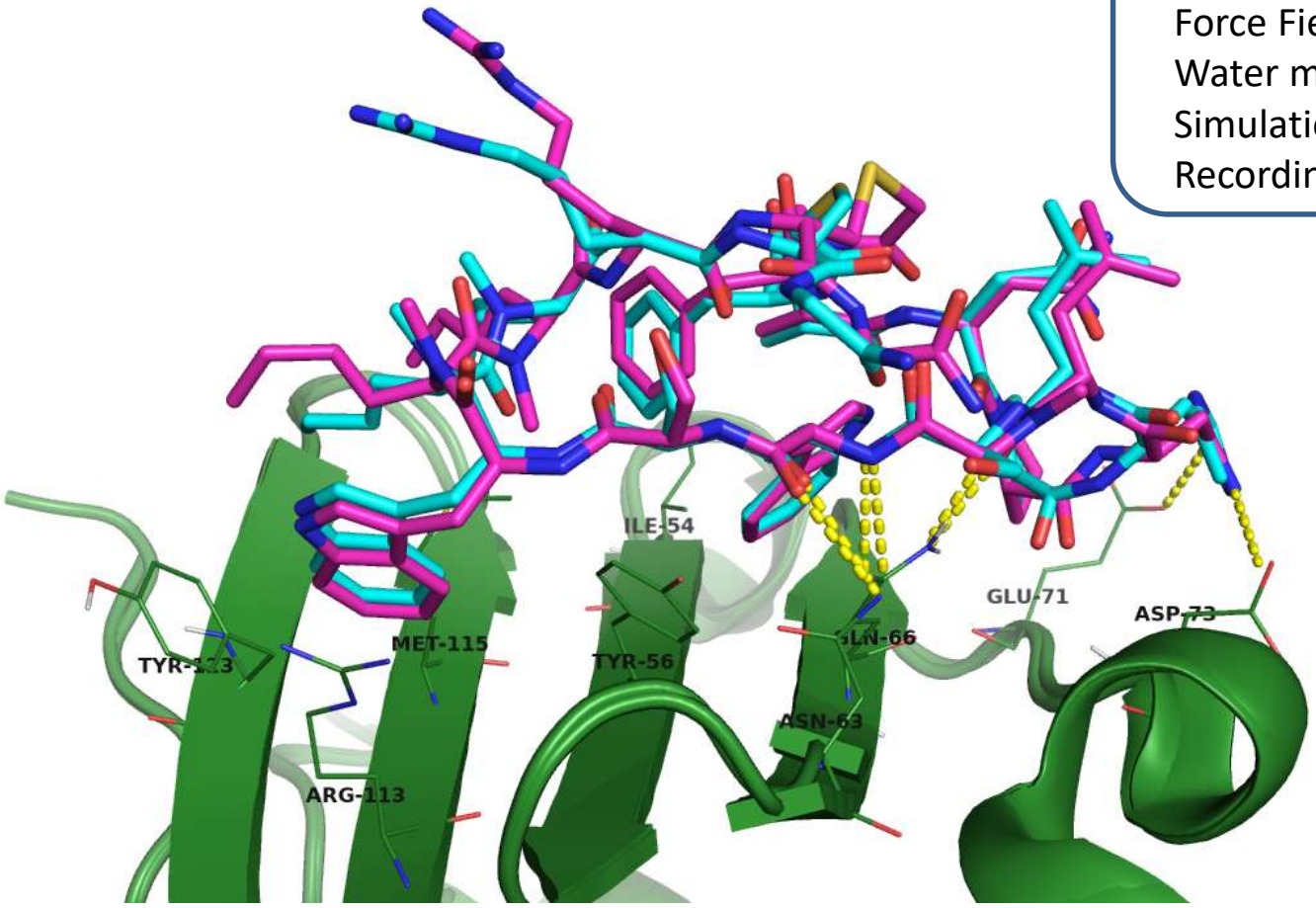


**6PV9**



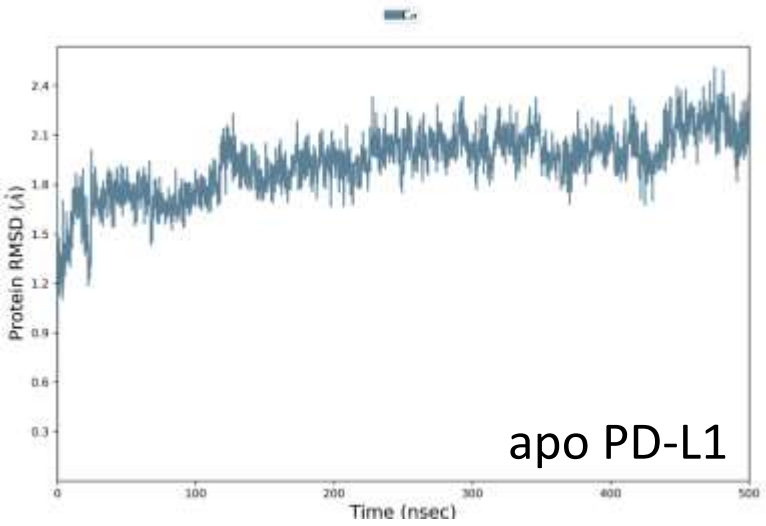
# PD-L1:macrocycle interactions

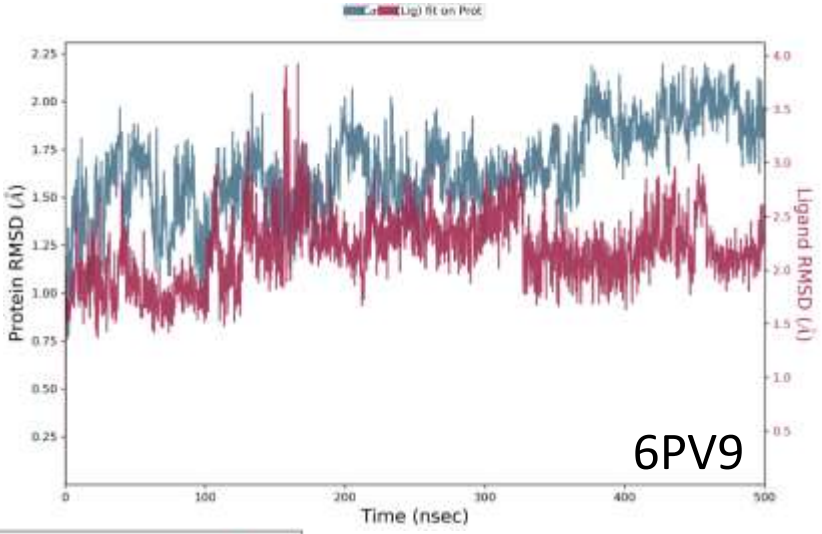
**MD simulation**
Desmond (Schrodinger*)
Force Field: OPLS3e
Water model TIP3P
Simulation time 500 ns
Recordin intervals: 250 ps

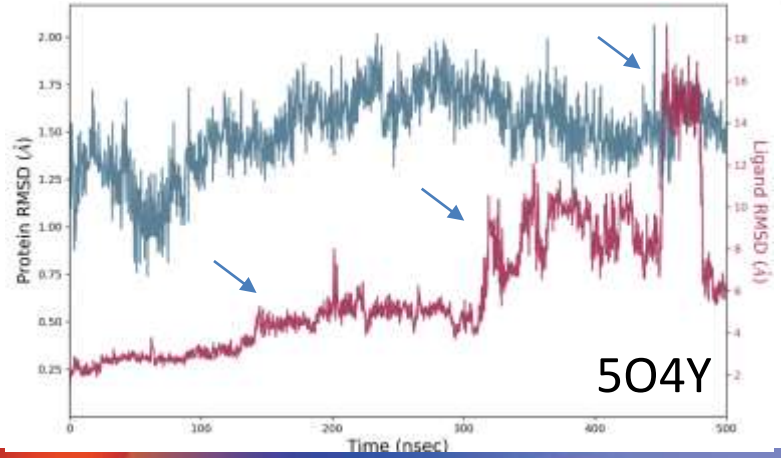



*Schrödinger Suite 2021-2, Schrödinger: New York, NY, USA, 2021.

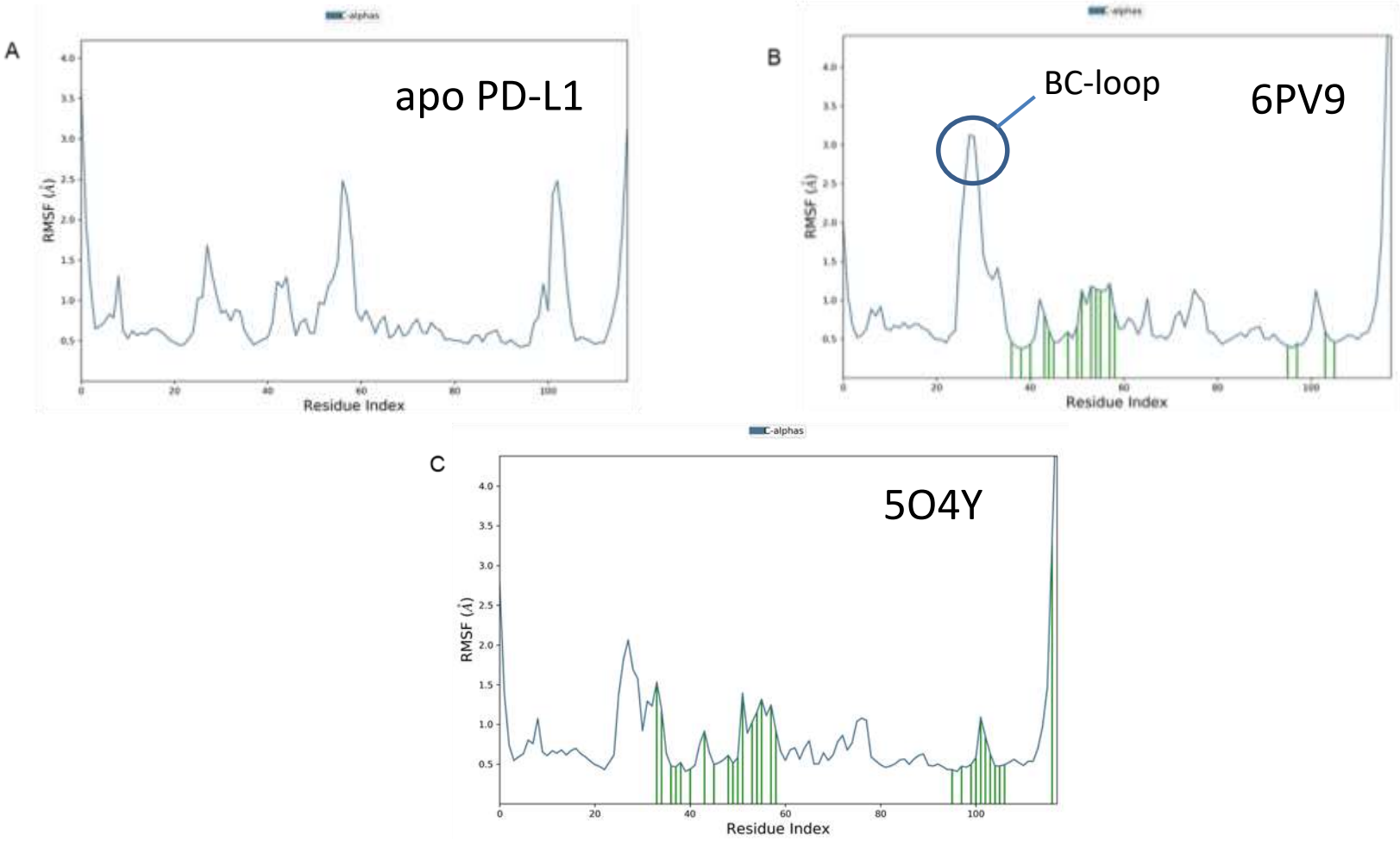
# MD simulation: RMSD analysis



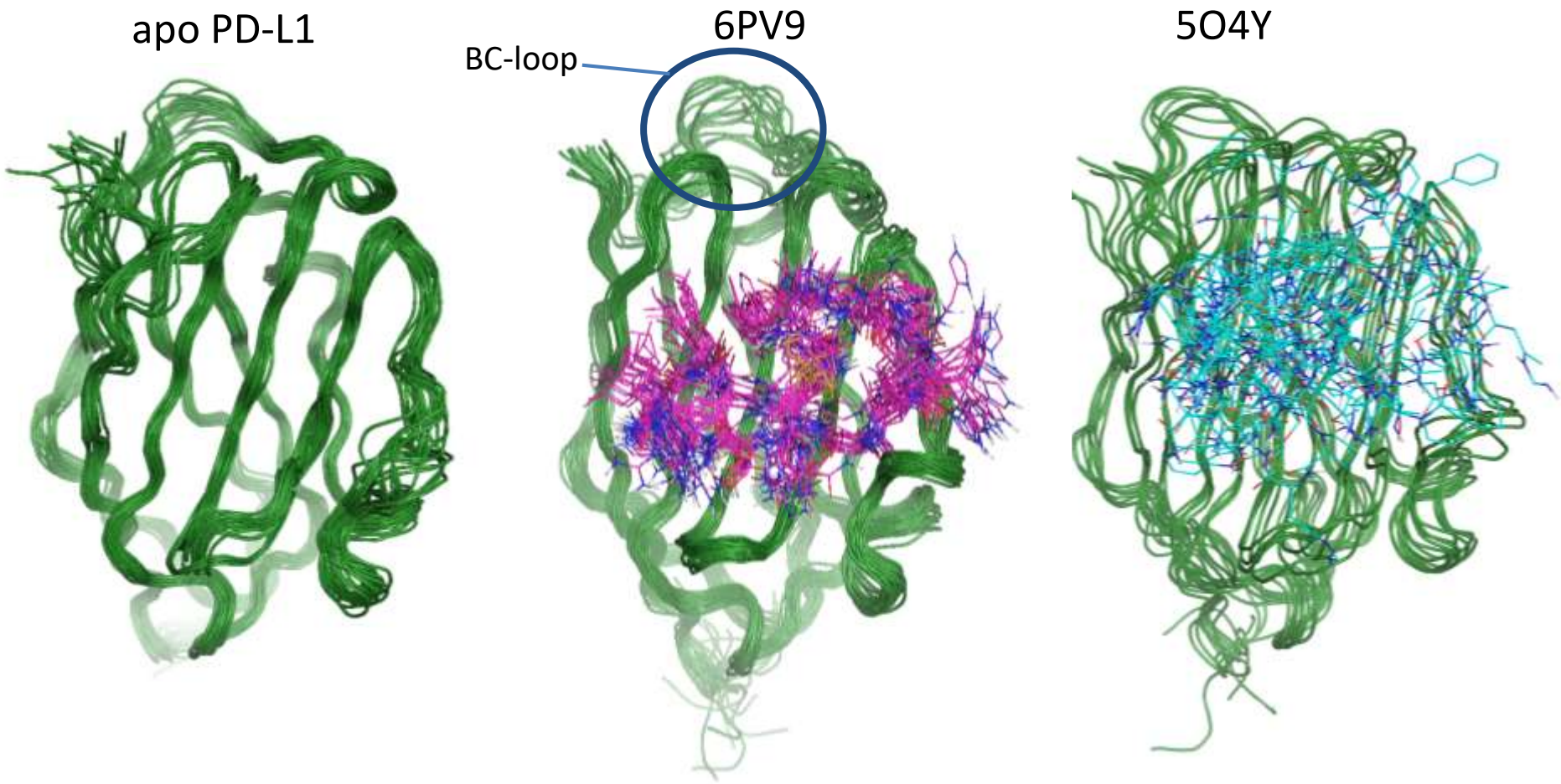



# MD simulation: RMSF analysis

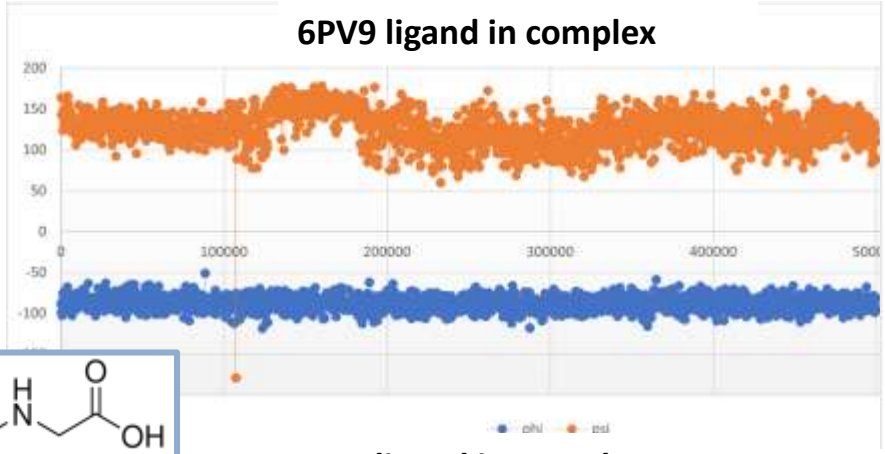


## Cluster representatives



# Sarcosine/serine dihedral angle analysis in free and bound ligands

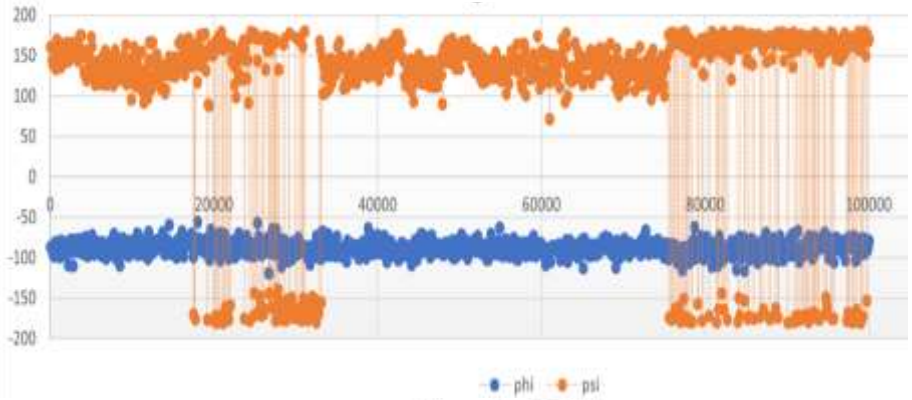
6PV9 ligand in complex



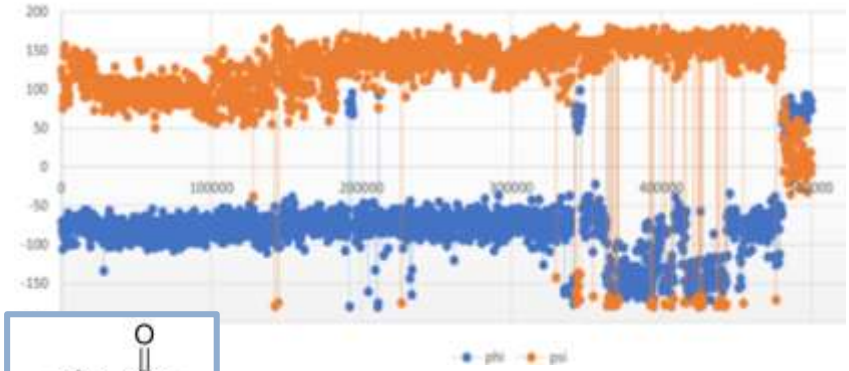


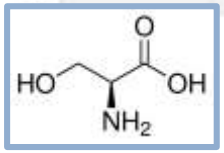


6PV9 ligand free

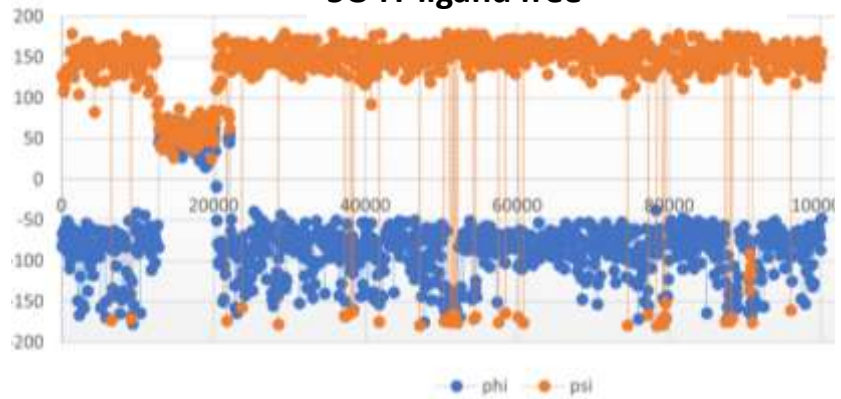



5O4Y ligand in complex



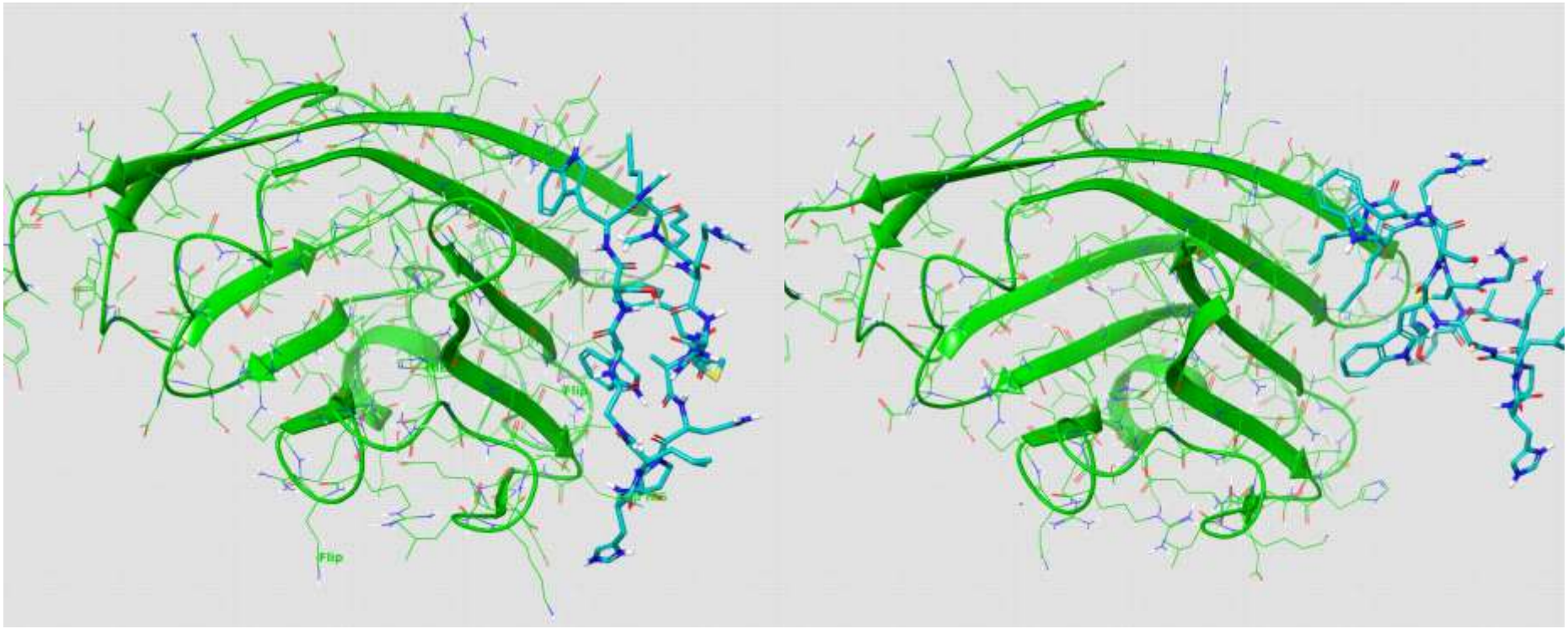




5O4Y ligand free



6PV9

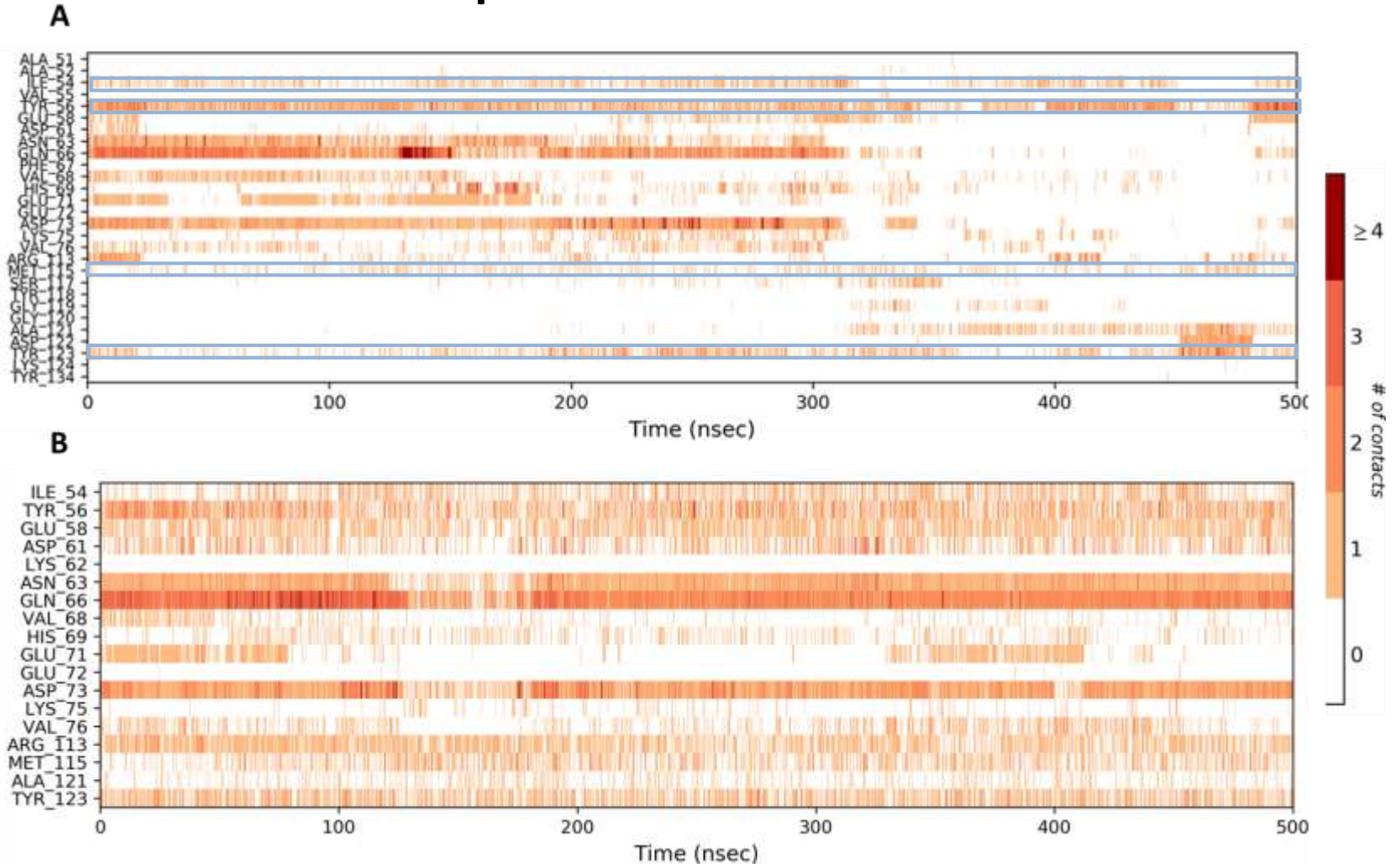
5O4Y



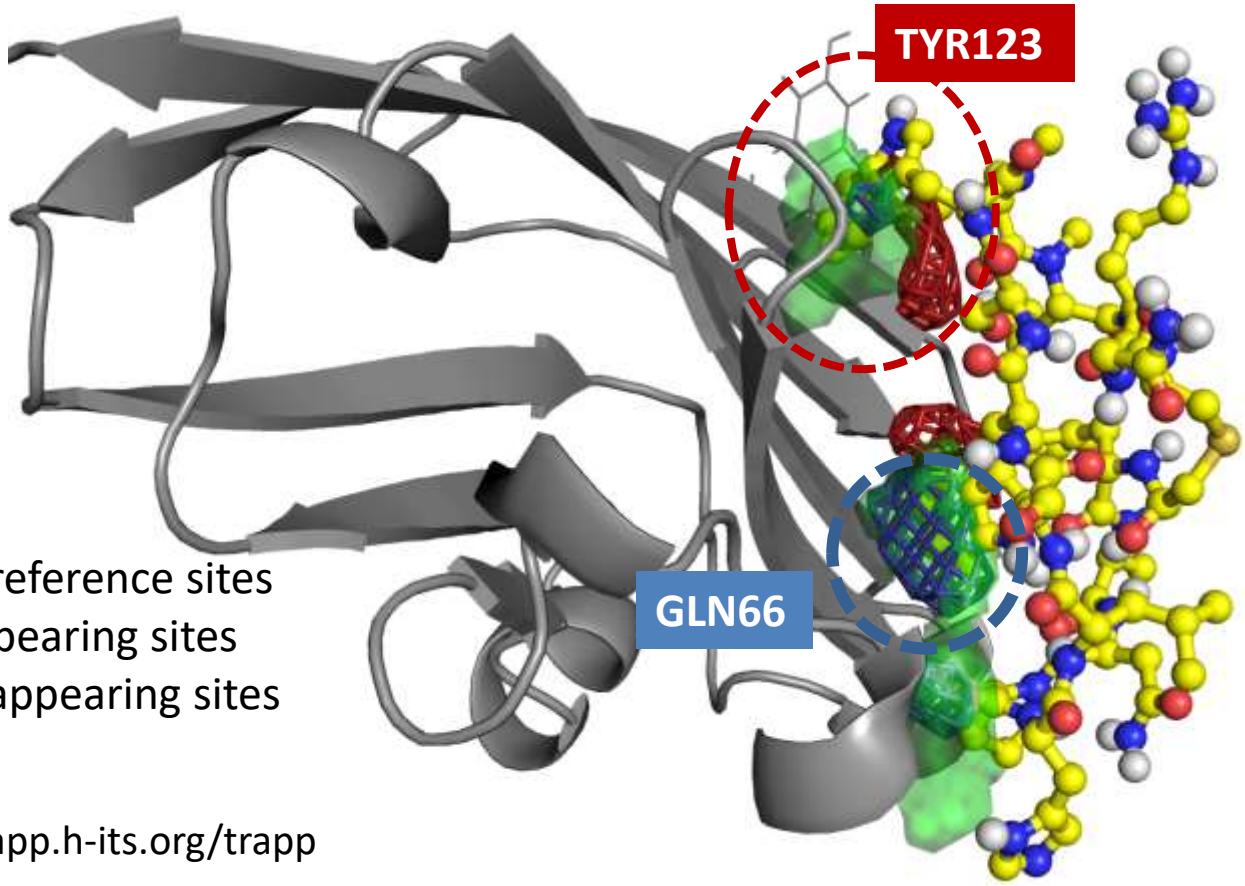
# PD-L1 residue interaction persistance

5O4Y

6PV9



# TRAnsient Pockets in Proteins (TRAPP)*




Green: reference sites
Red: appearing sites
Blu: disappearing sites

*https://trapp.h-its.org/trapp

# Conclusions

- ✓ The MD analysis highlighted as the most active ligand is also the most stable.
- ✓ The less active ligand maintain the hydrophobic contact with the hot spot of Tyr 123.
- ✓ The same hot spot was identified by TRAPP.
- ✓ The different stability can be attributed to the different conformational preferences of the mutated residue.

## Acknowledgments

Fabiana Pasquini

Marialuigia Fantacuzzi