ADVANCING RNA DRUG DISCOVERY THROUGH SHAMAN PROTOCOL

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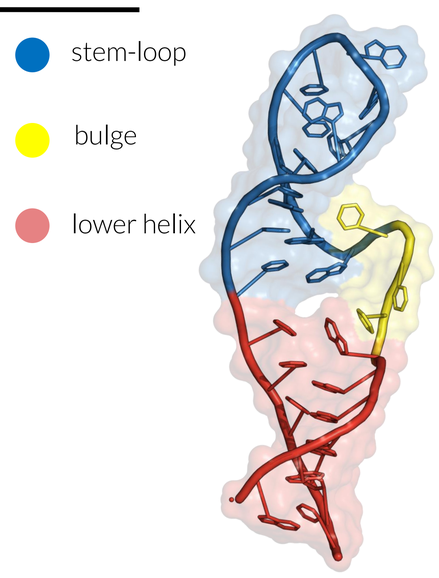
1. HIV-1 TAR as a pharmaceutical target

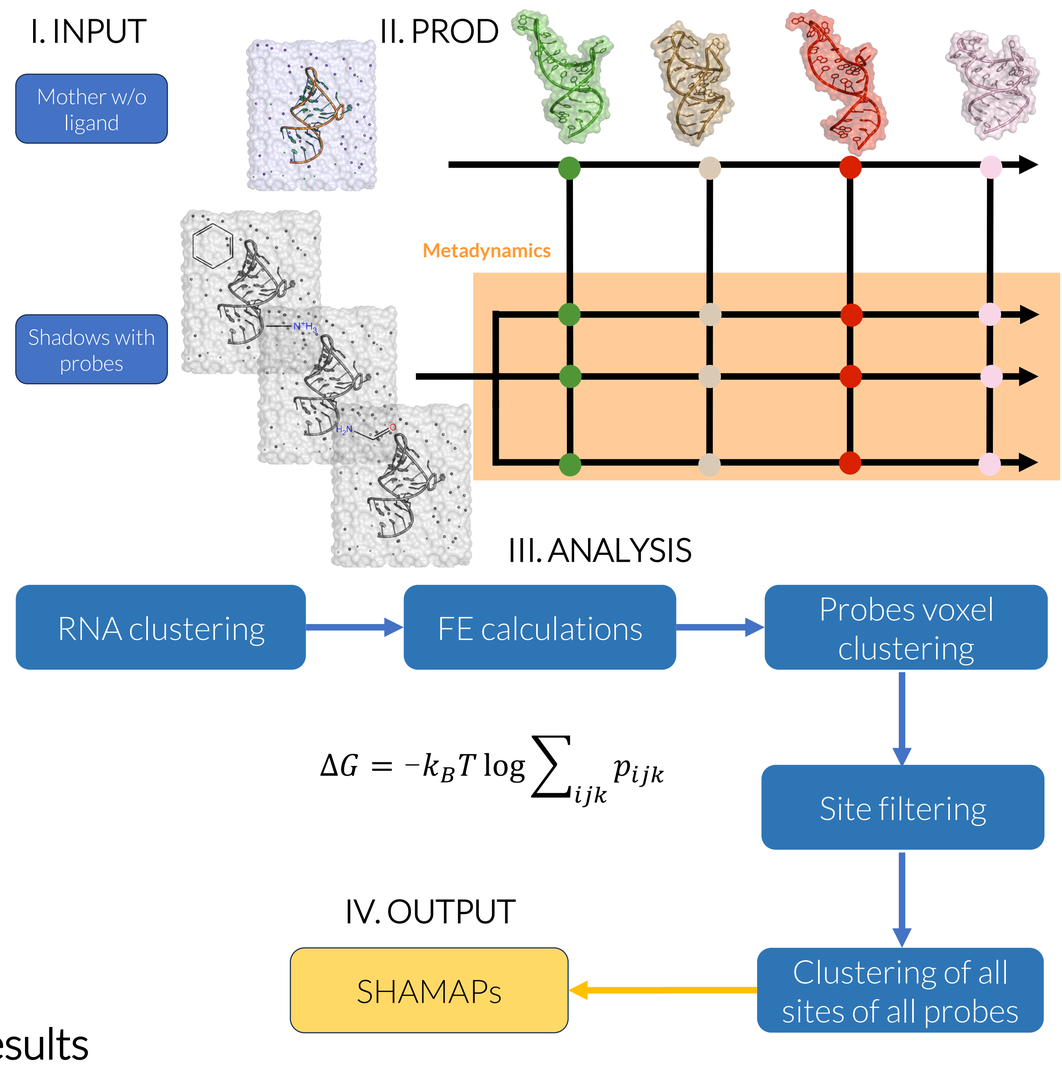
- The trans-activation response element (TAR) is an RNA stem-loop structure located at the 5' end of the HIV-1 genome [1].
- The conformation of the stem-loop is fundamental for the interaction with its biological partners, namely Integrase (IN) and Tat protein [2].
- There is no structure available of the TAR-IN complex.
- The aim of the project is to identify **disruptors** of TAR-IN interactions.



2. SHAMAN protocol

SHAdow Mixed solvent metAdyNamics (SHAMAN) [3] is a computational tool for binding site identification in dynamic RNA structural ensembles. The architecture shown below simultaneously allows for the following:

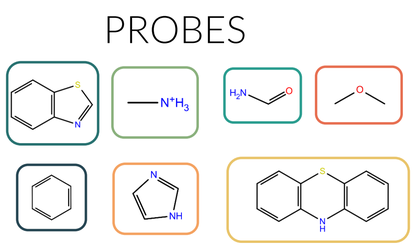
- Exploring **conformational landscape** with molecular dynamics (MD) simulations.
- Identifying potential **small-molecules binding site** with the aid of probes and Metadynamics.

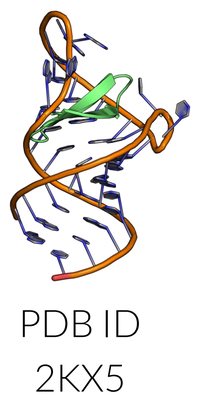


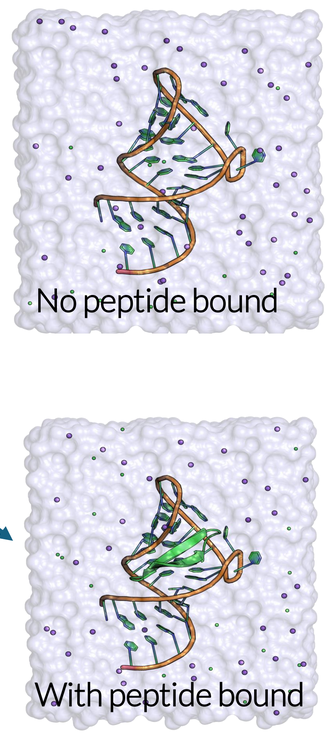
3. Systems setup

Since there is no available TAR-IN complex we decided to build our systems starting from a complex with a peptidomimetic of Tat (as it competes with IN).
We set up two different sets of simulations:

- Removing the peptide.
- Maintaining the peptide.





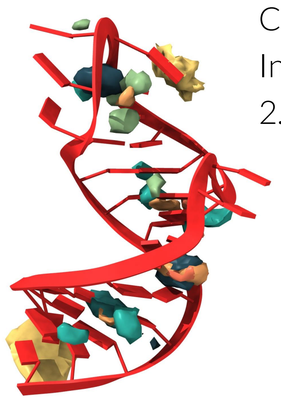


- 3 μs x replica
- RNA: ff99SB-ildn* + Parmbsc0 + χ_{OL3}
- Water: OPC
- Peptide: ff99SB
- SM: Sage 2.0

4. Preliminary results

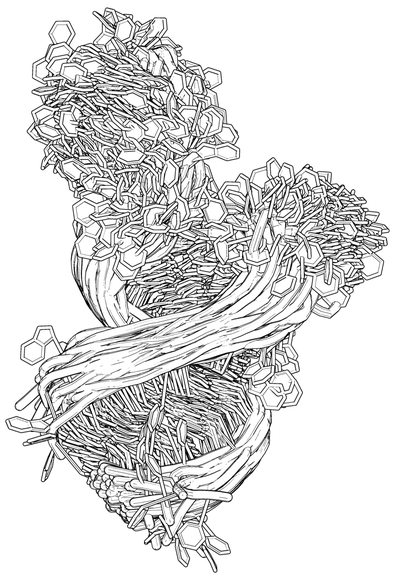
SHAMAPs

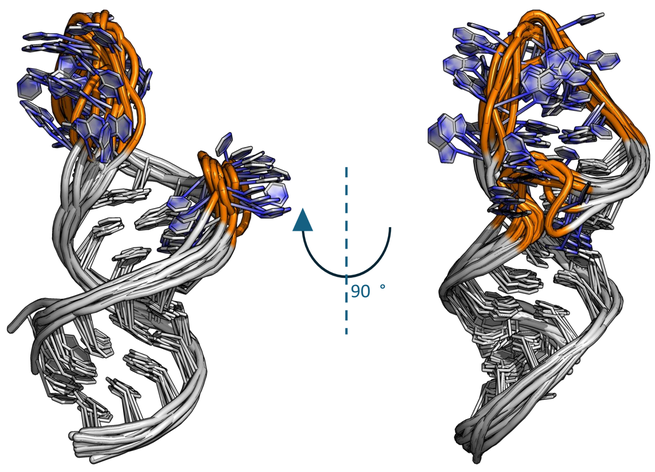
For each cluster representative, the output is a set of potential small molecule binding sites (SHAMAPs), each corresponding to a high-probability region occupied by at least one probe, ranked by binding free energy.



No peptide
Cluster 1 **89.7%**
Intracluster RMSD
2.74 Å

With peptide
Cluster 1 **98.6%**
Intracluster RMSD
2.63 Å

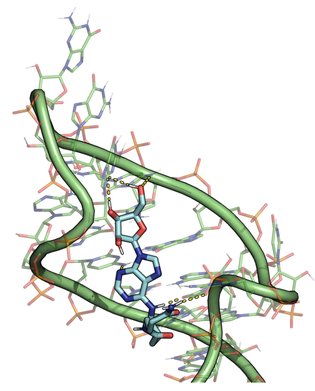




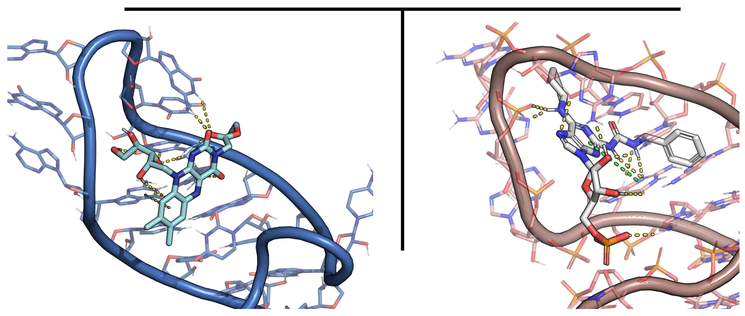
Due to the high intracluster structural variability, we decided to further cluster the structures into more refined subclusters using the following:

- **G-vectors** [4] as the metric.
- **QT clustering** [5] as the algorithm.

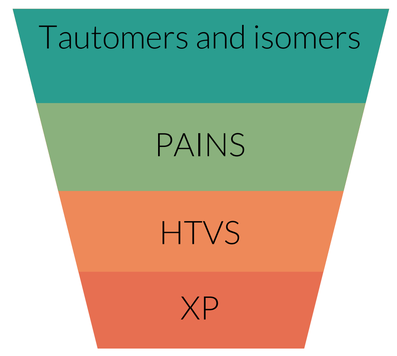
Virtual screening



8 subclusters were selected for virtual screening based on:
-**population**
-druggability score (**BiteNet**)
-compatibility with the **SHAMAPs**



ChemBioFrance



5. Perspectives

- 80 selected molecules will be experimentally tested as TAR-IN interaction disruptors.
- The use of G-vectors as metrics to refine the cluster analysis in SHAMAN will be integrated into the pipeline.
- A novel version of SHAMAN will be developed to better explore conformational flexibility

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References:
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[2] Rocchi C, et al. International Journal of Molecular Sciences. 2022;23(22):13742.
[3] Panei, F. P., et al. Nature Communications 2024;15(1): 5725.
[4] Bottaro, S., et al. Nucleic acids research 2014;21(42):13306-14.
[5]González-Alemán, R., et al. Journal of chemical information and modeling 2019;60(2): 467-472.