COMPARING LIGAND BINDING TO PROTEIN AND RNA TARGETS: THE CASE OF RIBOFLAVIN

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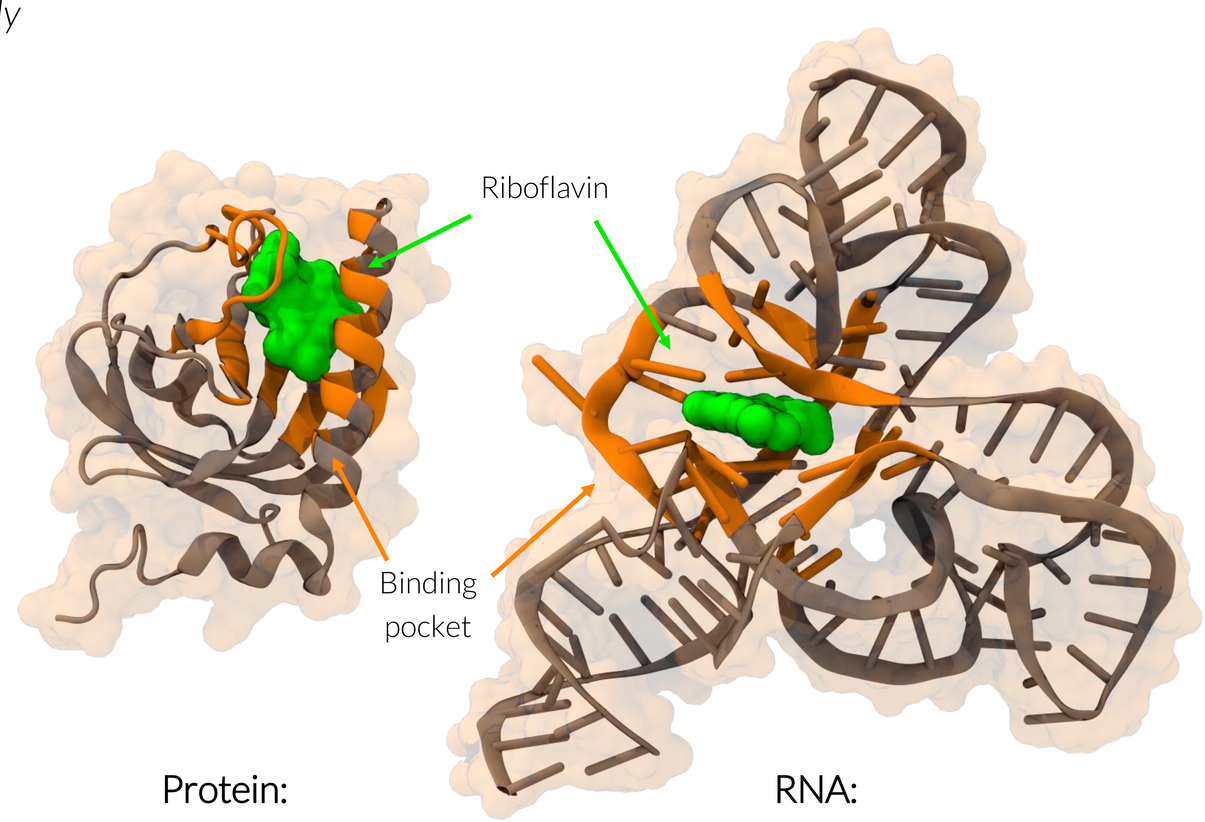
Introduction

- **Non-coding RNA** molecules play essential roles in many biological processes. These RNAs can adopt highly structured conformations, suitable for binding pocket formation [1,2];
- RNAs are challenging targets for standard **drug discovery** due to their complex dynamics and electrostatic properties [3];
- In this study, we focused on key features of drug-target binding, studying the similarities and differences existing between protein and RNA ligand binding;
- We selected as a test case riboflavin, which binds both protein and RNA partners: **riboflavin kinase** and **flavin mononucleotide** (FMN) **riboswitch**.



Protein:
Riboflavin kinase
PDB-ID: 1NB9 [4]

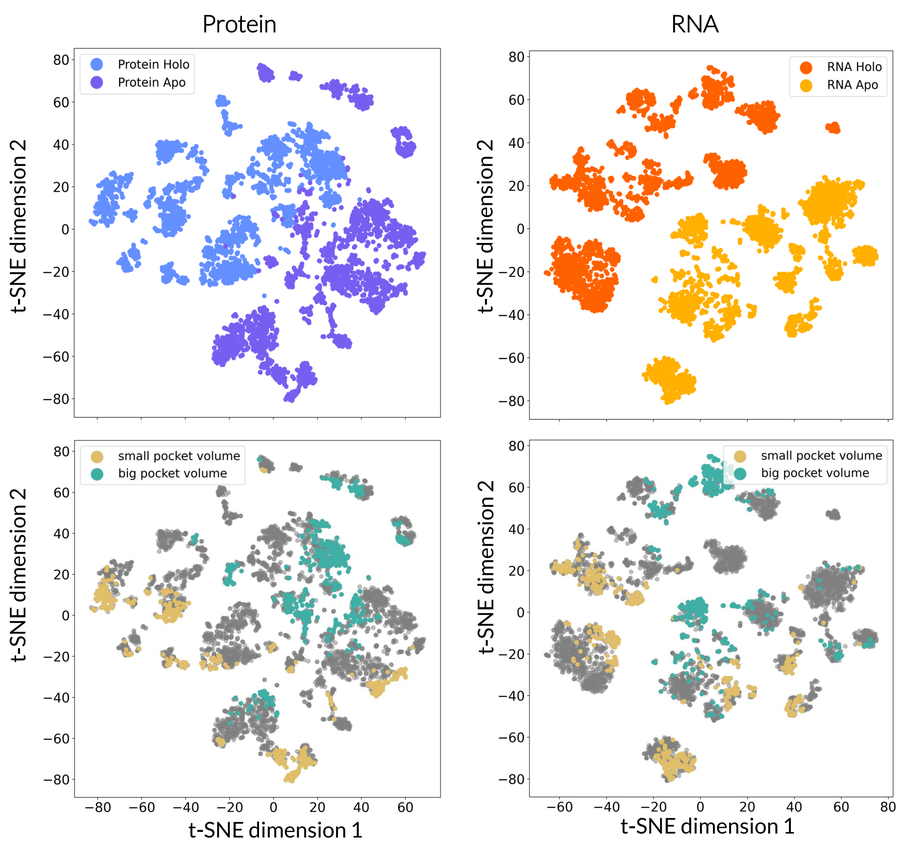
RNA:
FMN riboswitch
PDB-ID: 3F4G [5]

Conformational flexibility

We analyzed the structural features of the riboflavin binding site:

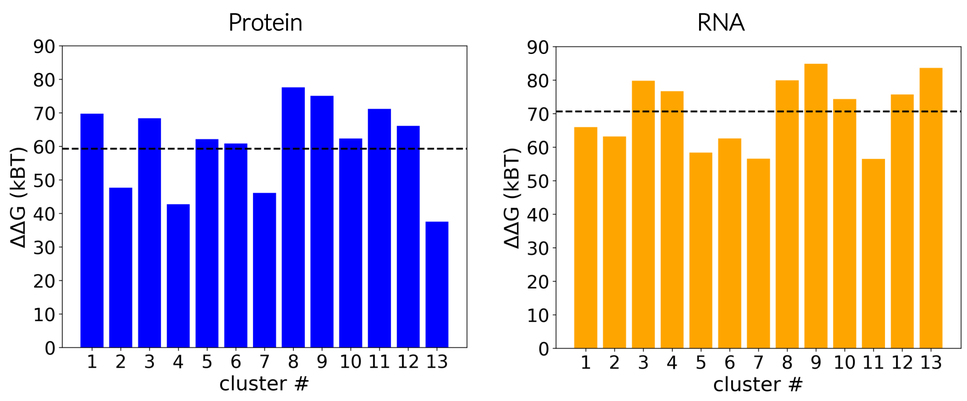
- Distribution of the pocket volume;
- Pocket conformational space.

To characterize the **pocket conformational spaces**, we monitored the minimum distances between the residues comprised in the pockets and applied dimensional reduction techniques (PCA, **t-SNE**).



- Protein displays overlap in the conformational space of the Apo and Holo systems, in RNA the separation is sharp;
- Pockets with similar conformations have also comparable volumes.

Electrostatic contribution to ligand binding



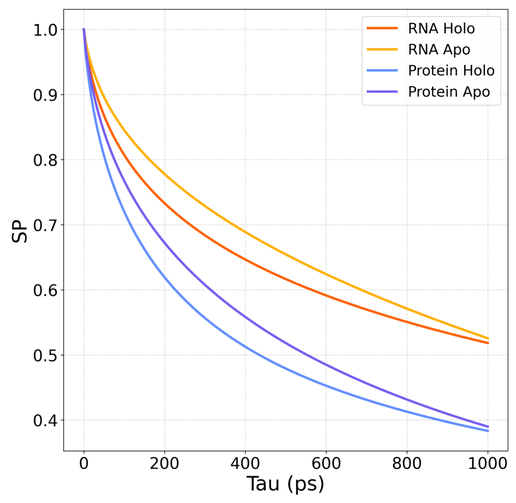
- Delphi [6] was used to perform calculations on the centroids extracted through k-means clustering on the t-SNE spaces;
- Both the systems show positive and equivalent terms for the electrostatic contribution to the binding free energy.

Water dynamics

Water residence time

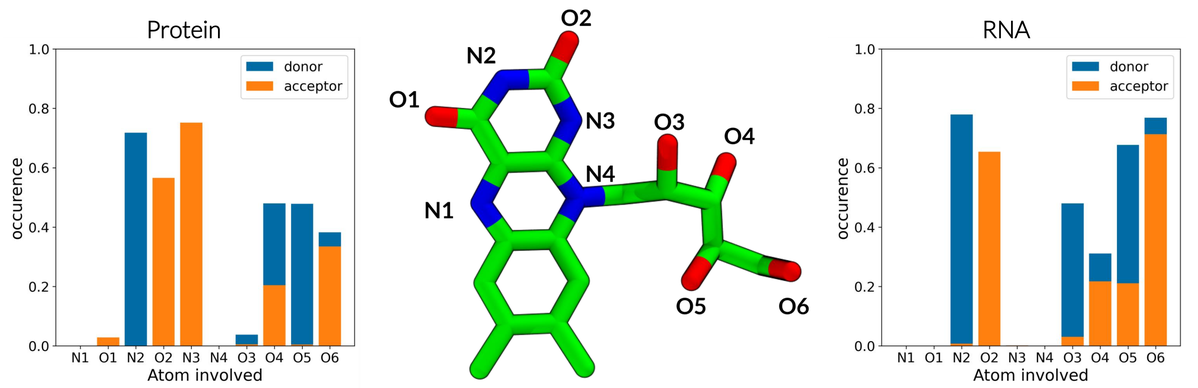
To investigate this property, we used the **survival probability** (SP). In this case, we define a spherical region centered in the pocket to monitor the survival of water molecules.

$$SP(\tau) = \frac{1}{T}\sum_{t=1}^{T}\frac{N(t, t+\tau)}{N(\tau)}$$



- τ_{max}=1 ns and intermittency of 10 ps (2 frames), as implemented in MDAnalysis [7];
- Water residence time is smaller in the protein binding pocket.

H-bond analysis



- A larger number of H-bonds involving the ligand head in the protein, a larger number involving the ligand tail in RNA;
- Comparable donor/acceptor ratio in both protein and RNA.

Conclusions

- The structural behaviors appeared rather similar, the RNA molecule explored **different conformations** and showed **enhanced fluctuations**;
- The electrostatic term of the binding free energy seems equivalent for the systems;
- The RNA binding pocket formed multiple hydrogen bonds with the ligand, similar to the protein target, highlighting the possibility to fulfill a **specific interaction network**;
- The RNA binding pocket appeared **more solvated**, which may be a peculiar feature of these binding sites.

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