Analyzing switch regions of human Rab7a and Rab10 by molecular dynamics simulations

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PROFESSIONAL TRAJECTORY

Levy Bueno Alves

- **2015-2019**: Degree in Biotechnology from the Federal University of Alfenas;
- **2020-2022**: Master's Degree in Genetics from the University of São Paulo;
- **2022**: PhD student in Genetics from the University of São Paulo.

INTRODUCTION

Rab7a and Rab10 targets

- Belongs to the family of Rab GTPases;
- It is responsible for regulating intracellular traffic in various pathways of different cellular sublocations;
- Functional deregulation in the pathways of theses enzymes are implicated in ciliopathies, cancer and neurodegenerative diseases.

Figure 1. Rab7a and Rab10 cellular sub-locations

Endosplasmatic Reticulum

Trans-Golgi Network

Endosomes

Lysosomes

Primary cilium

Reference: Author

INTRODUCTION

Rab7a and **Rab10** in **Alzheimer’s disease**

- Studies have shown that these enzymes play a relevant role in Alzheimer's disease.
  - Rab10 helps in the **amyloidogenic processing** of the amyloid precursor protein (APP);
  - Rab7a is involved in the **hyperphosphorylation of the Tau protein**.

References: P. G. RIDGE et al., 2017; L. Rodriguez et al., 2017.

Rab7a and Rab10 in cancer

- Studies that correlate the inhibition of these enzymes with the induction of apoptosis in cancer cells;
  - These enzymes can activate signaling pathways for **cancer cells growth and survival**, leading to cancer progression.

References: G. D. P. Krishnan et al., 2021.

INTRODUCTION

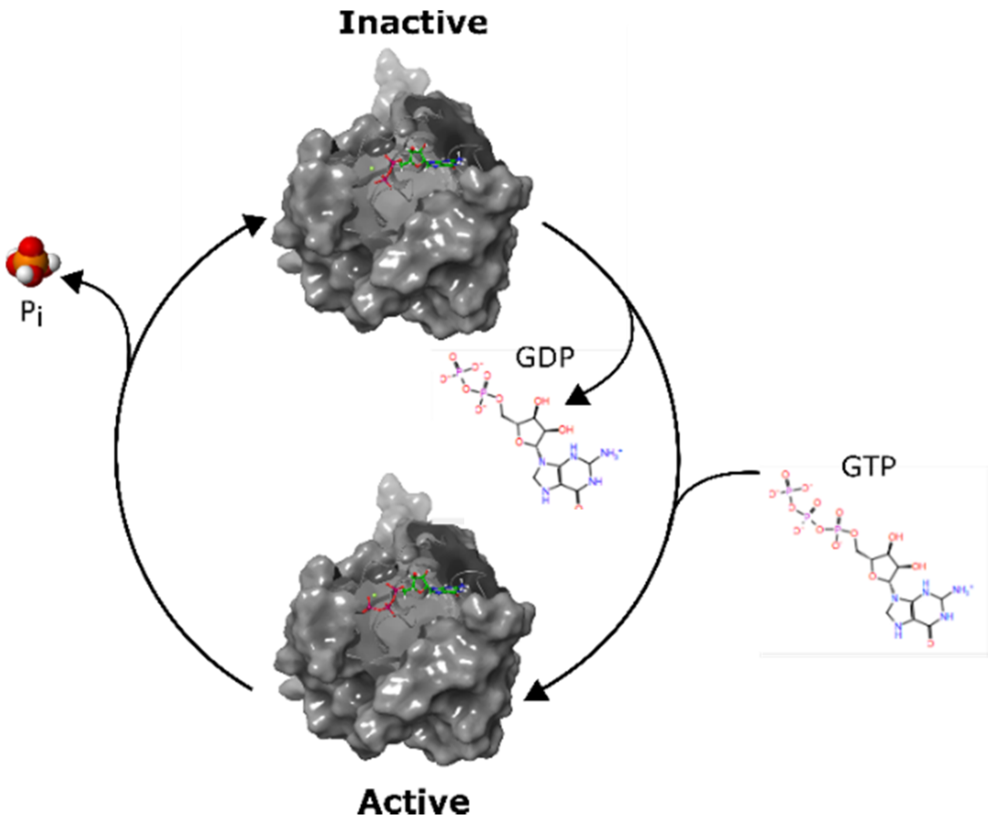
Therapeutic application

- Such evidences pave the way for the application of new drug targeting strategies for the treatment of various human diseases:
  - The **modulation of the activity of these enzymes** using small molecules may represent a promising alternative to delay the progression of these diseases.

Cycle of small GTPases

- Rab GTPases regulate cellular processes by alternating the nucleotides **GTP** and **GDP**;
  - When bound to GTP, these enzymes are in active state.
  - When bound to GDP, these enzymes are in inactive state.
- The **switch 1** and **switch 2** regions:
  - When activated, it has ⬇ flexibility;
  - When inactivated, it has ⬆ flexibility;

Figure 2. The GTP → GDP cycle of small GTPases

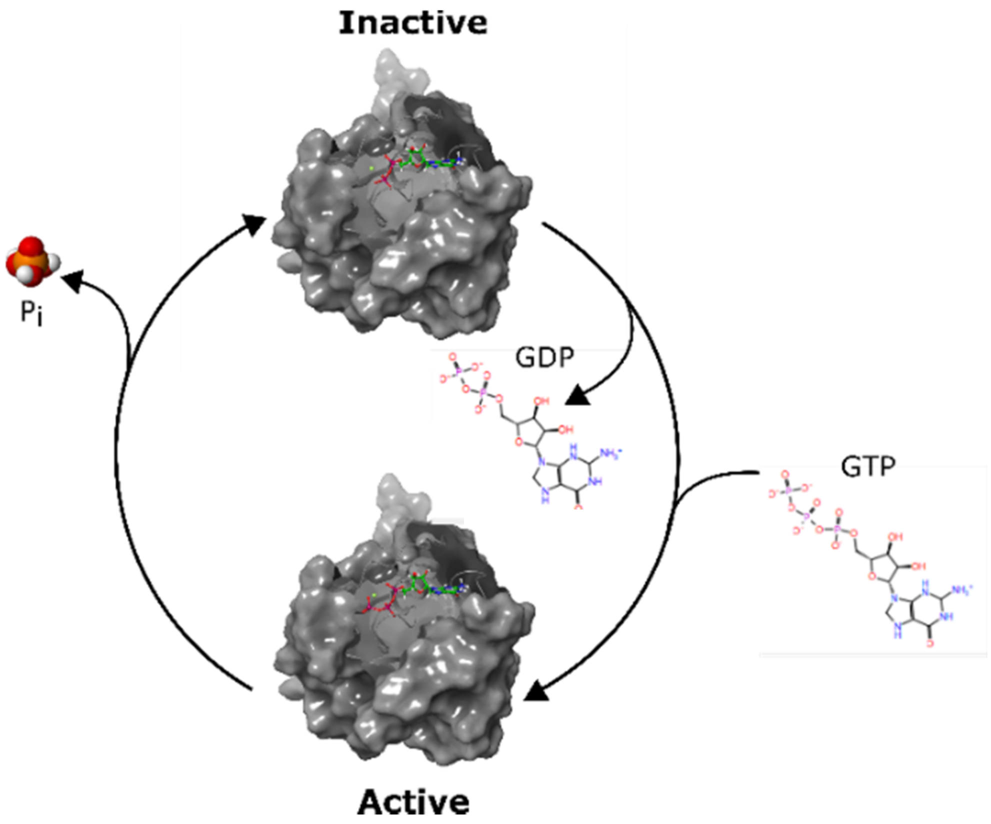


Reference: Author

Cycle of small GTPases

Figure 2. The GTP → GDP cycle of small GTPases

- Interactions with the phosphate-binding motifs (PMs):
  - **GTP** interacts with PM1, PM2 and PM3;
  - **GDP** interacts only with PM1.



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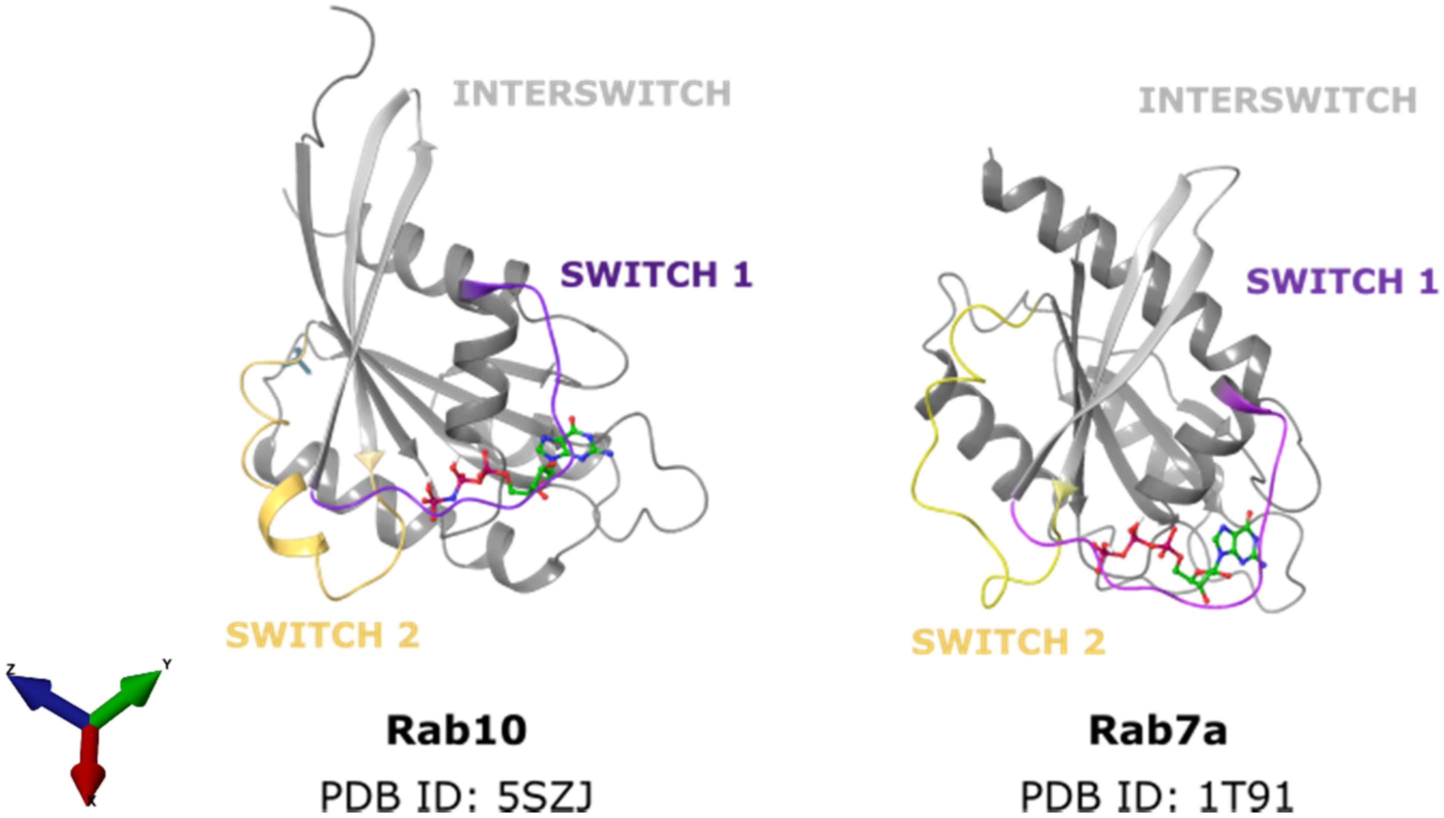
OBJECTIVE

- The present study aimed to detail the **structural flexibility** of the switch regions of Rab7a and Rab10 by molecular dynamics (MD) simulations.

Figure 3. Rab10 switch 1, interswitch and switch 2 regions



Reference: Author

OBJECTIVE

- The present study aimed to detail the **structural flexibility** of the switch regions of Rab7a and Rab10 by molecular dynamics (MD) simulations.

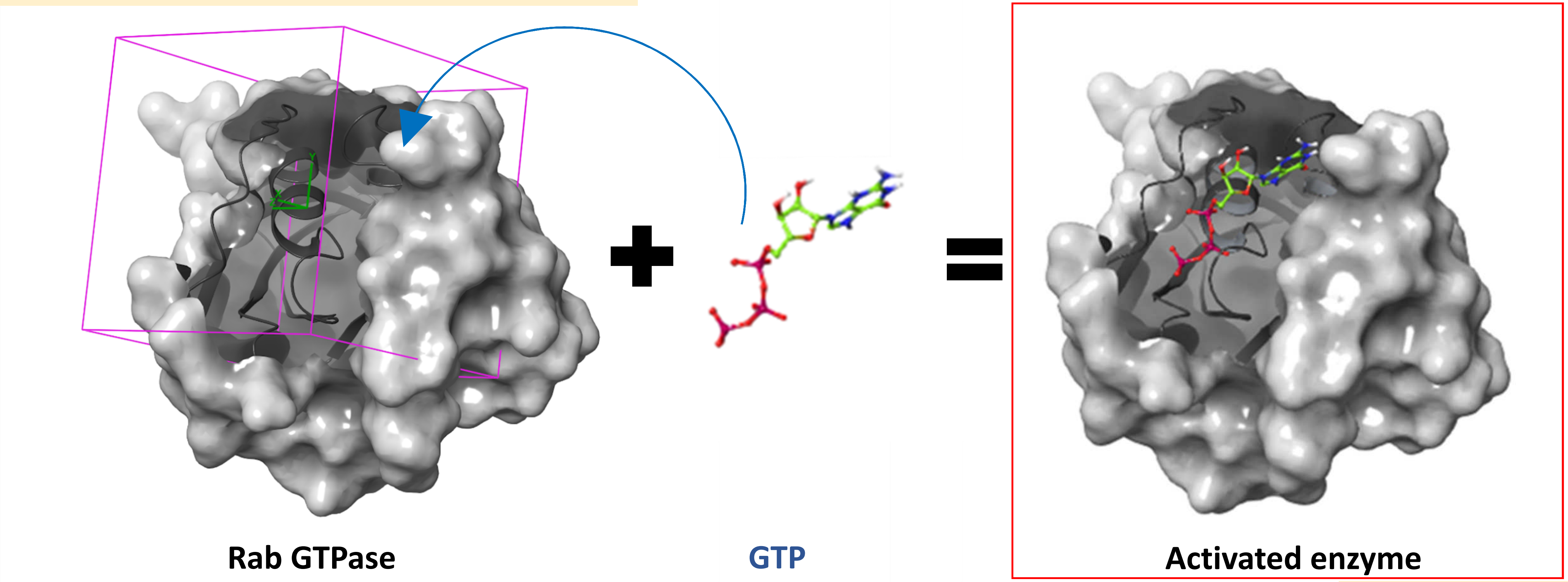
- **2020:** our research group performed MD simulations to investigate the internal movements of Rab10.
    - These results showed noticeable differences in local flexibility of switch1 when Rab10 was bound with GDP.
    - In order to verify if the conformational change in the on-to-off process of Rab10 was not by chance, we extended our study considering Rab7a.

METHODS

Molecular docking

- We used molecular docking to form complexes with **GDP** and **GTP**.

Figure 4. GTP docking at Rab's active site

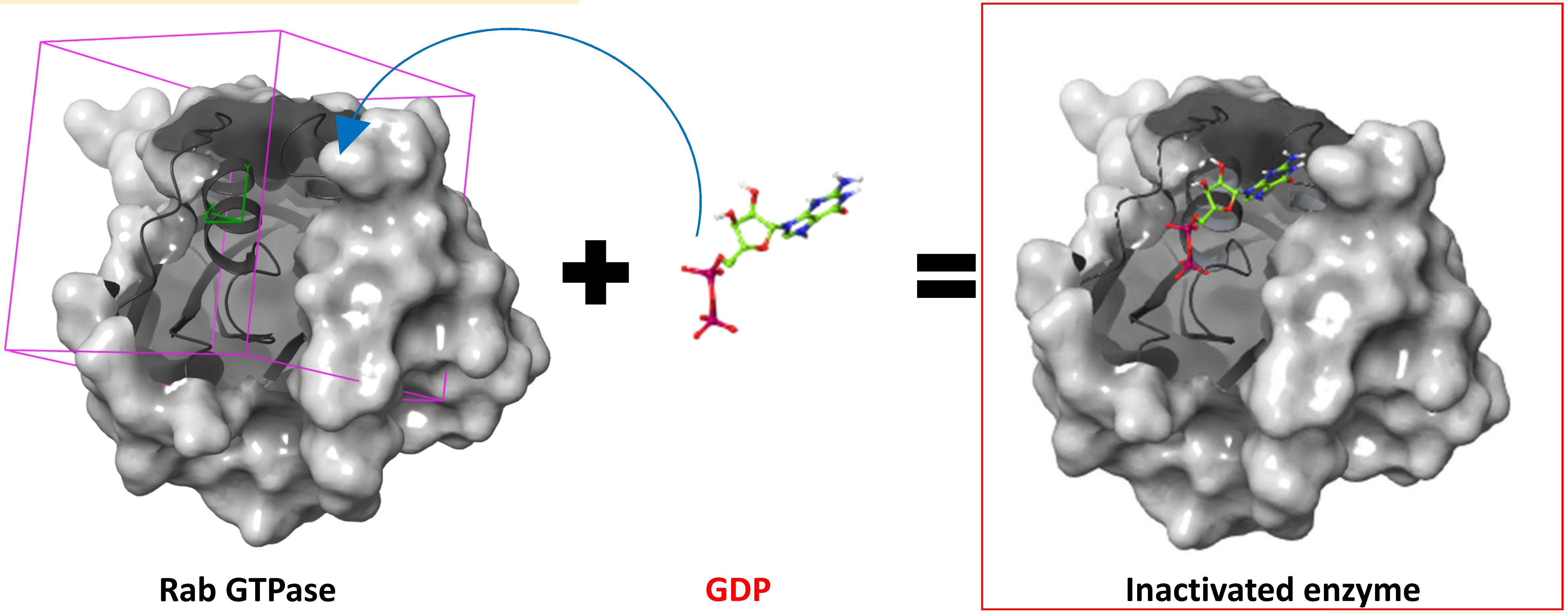


Reference: Author

Molecular docking

- We used molecular docking to form complexes with GDP and GTP.

Figure 5. GDP docking at Rab's active site

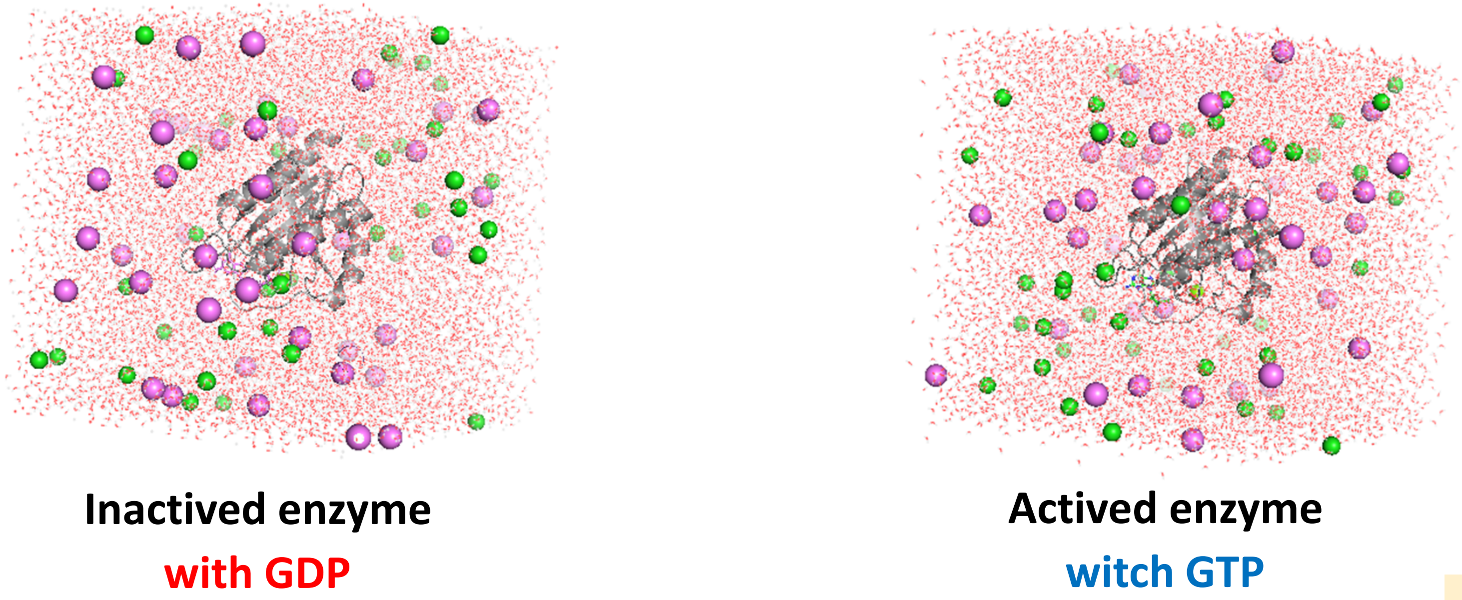


Reference: Author

Molecular dynamics (MD)

- The root mean square deviation **(RMSD)**, root mean square fluctuation **(RMSF)**, radius of gyration **(Rg)** and solvent accessible surface area **(SASA)** were used for the trajectory analysis.

Figure 6. Inactived and actived systems



Inactived enzyme
with GDP

Actived enzyme
witch GTP

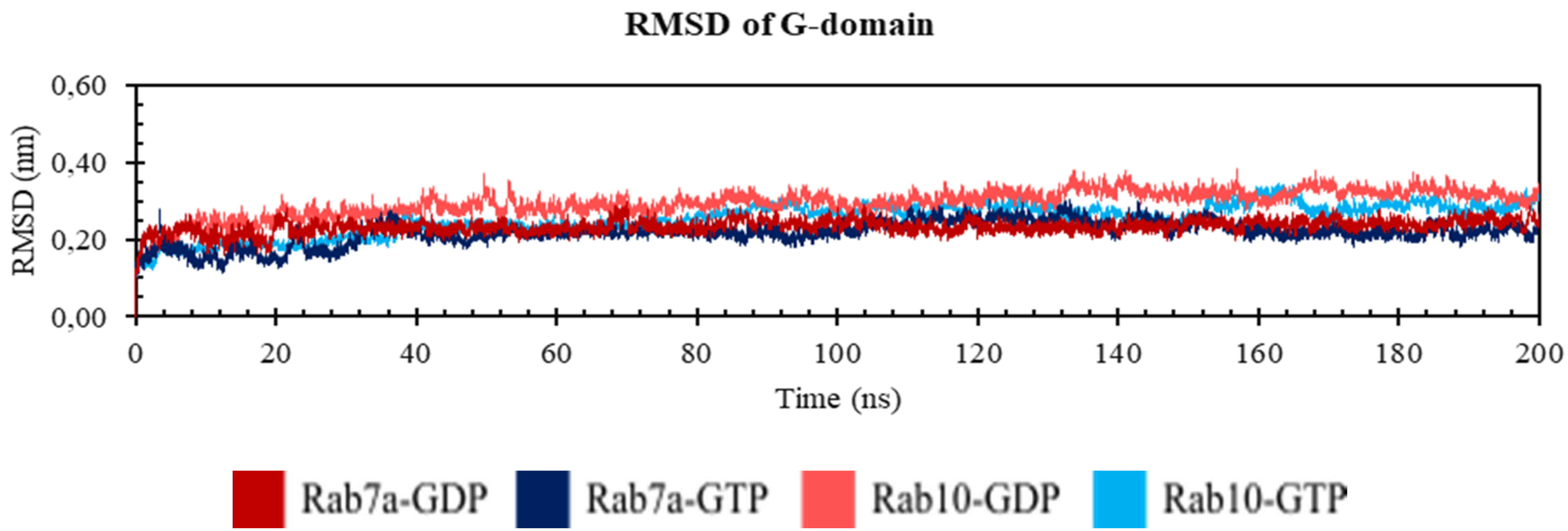
Reference: Author

RESULTS AND DISCUSSION

Molecular dynamics study

- **RMSD analysis:** the trajectories reached stability after 40 ns of simulation. The complexes associated with the GTP have more restricted movements.

Figure 7. G-domain RMSD of Rab7a and Rab10.



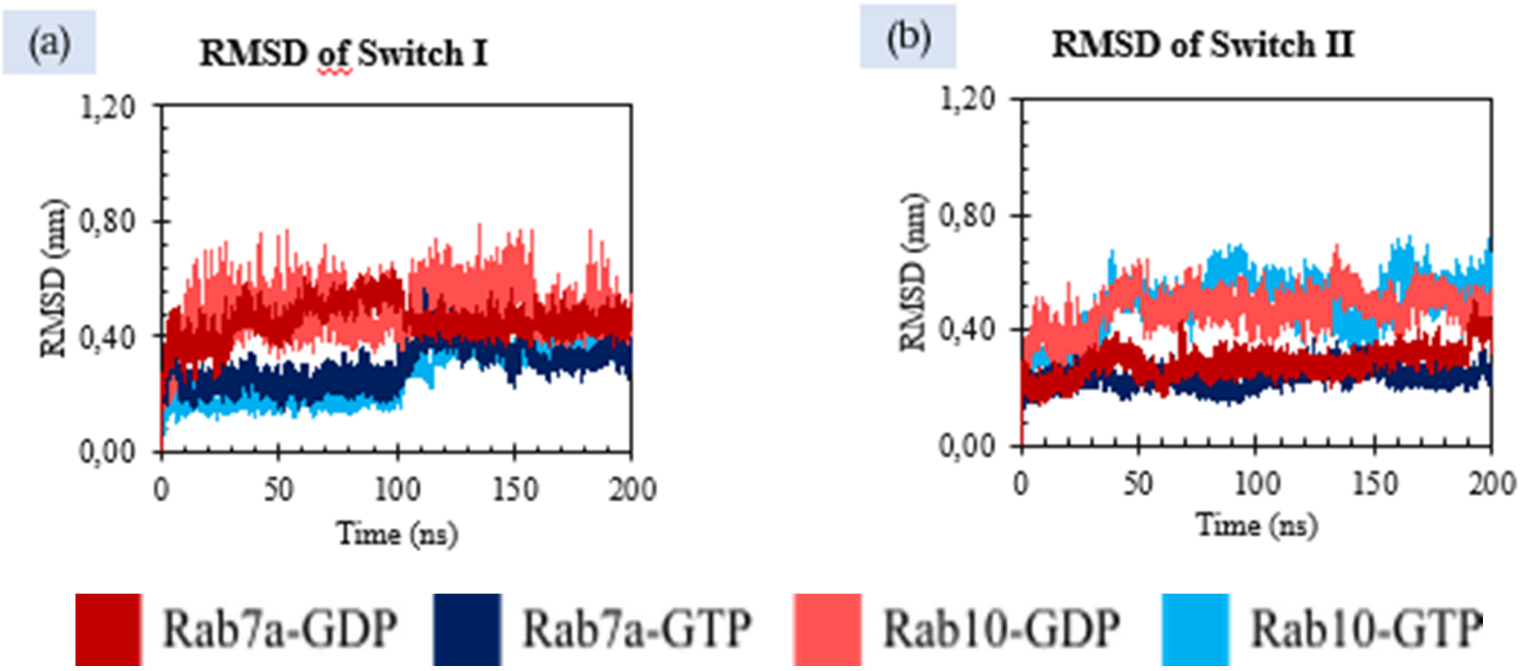
Reference: Author

RESULTS AND DISCUSSION

Molecular dynamics study

- **RMSD analysis:** switch 1 region showed significant differences when the Rab's was associated with the tested nucleotides.

Figure 7. RMSD of switch regions of Rab7a and Rab10: (a) RMSD of Switch 1 and (b) RMSD of Switch 2.



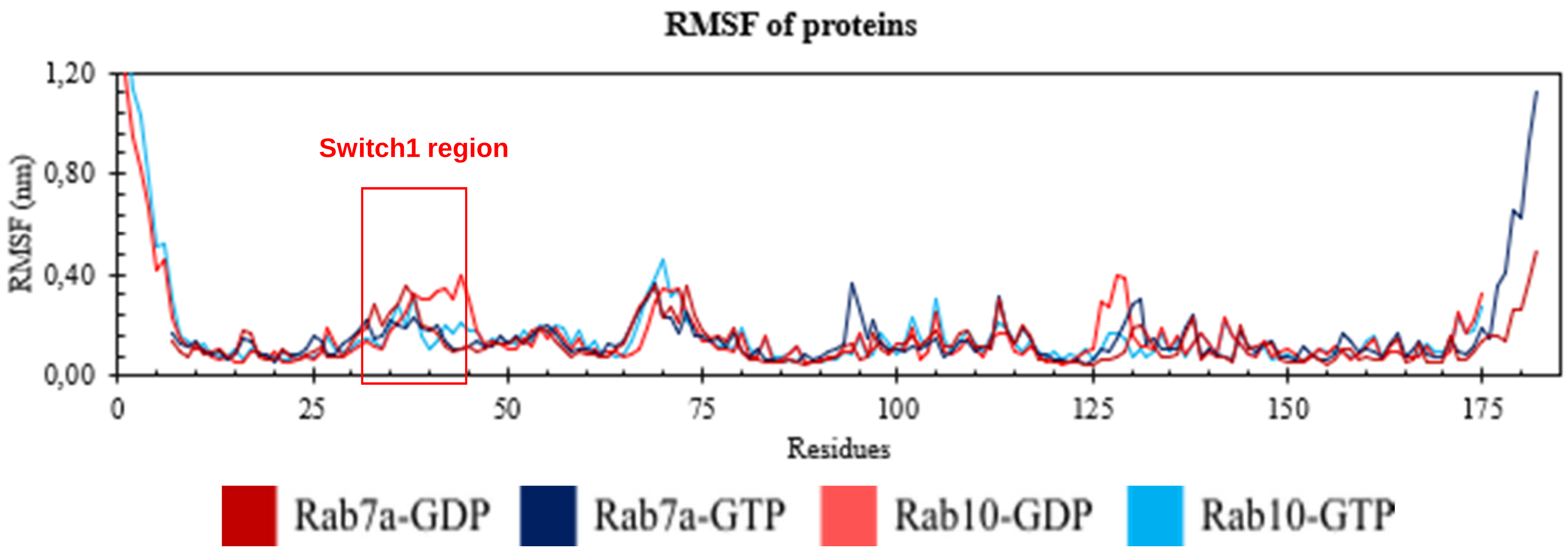
Reference: Author

RESULTS AND DISCUSSION

Molecular dynamics study

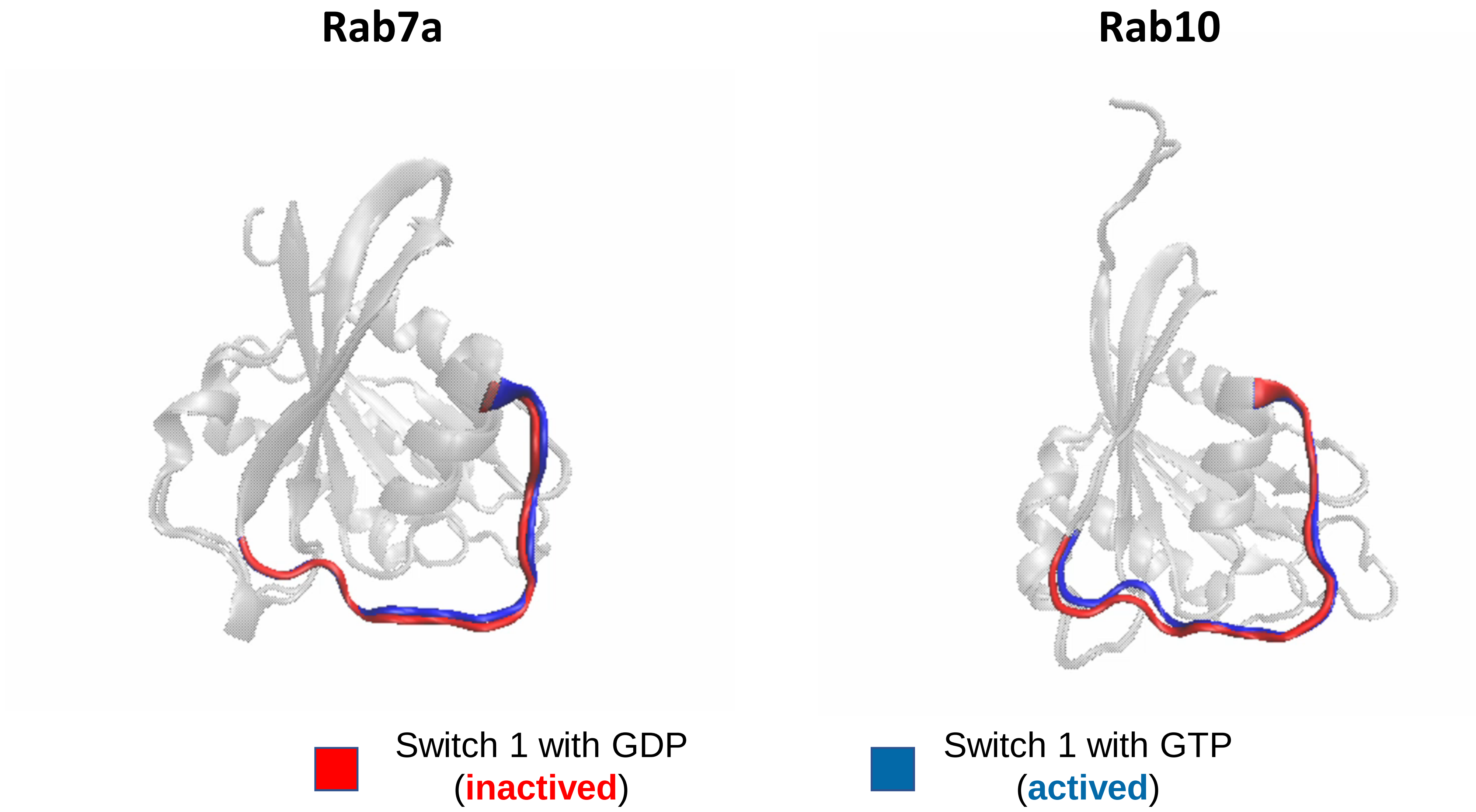
- **RMSF analysis:** The residues composes the switch 1 region showed greater fluctuations when the Rab’s is linked to GDP.

Figure 8. RMSD of switch regions of Rab7a and Rab10: (a) RMSD of Switch 1 and (b) RMSD of Switch 2.



Reference: Author

RESULTS AND DISCUSSION



🟥 Switch 1 with GDP (**inactived**)

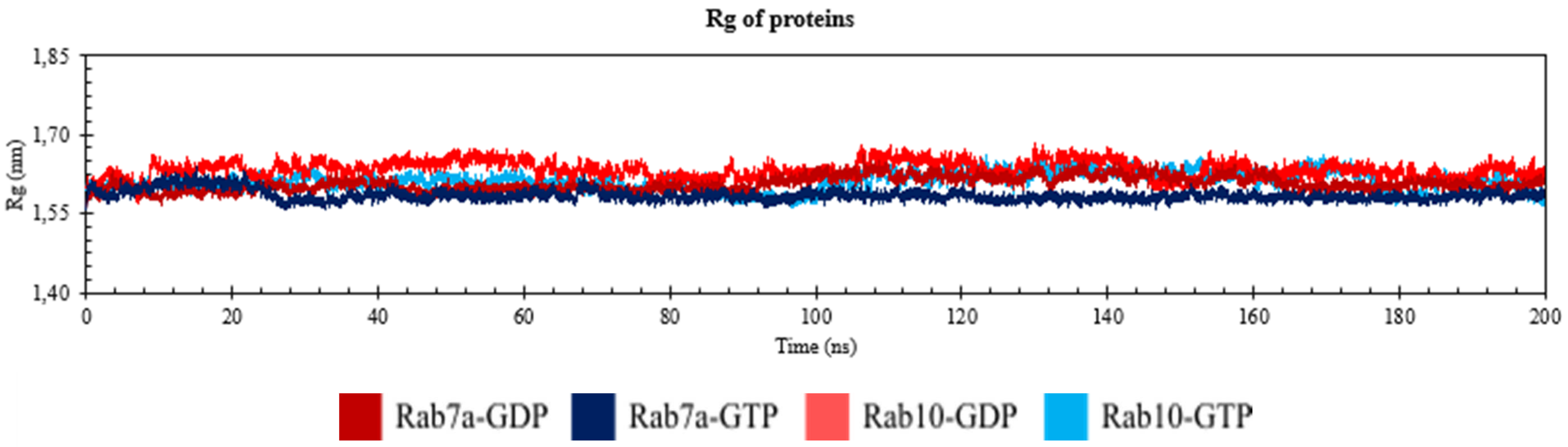
🟦 Switch 1 with GTP (**actived**)

RESULTS AND DISCUSSION

Molecular dynamics study

- **Rg analysis:** the Rg trajectories of the inactivated systems showed higher values than the activated state.

Figure 9. Rg of G-domain of Rab7a and Rab10.

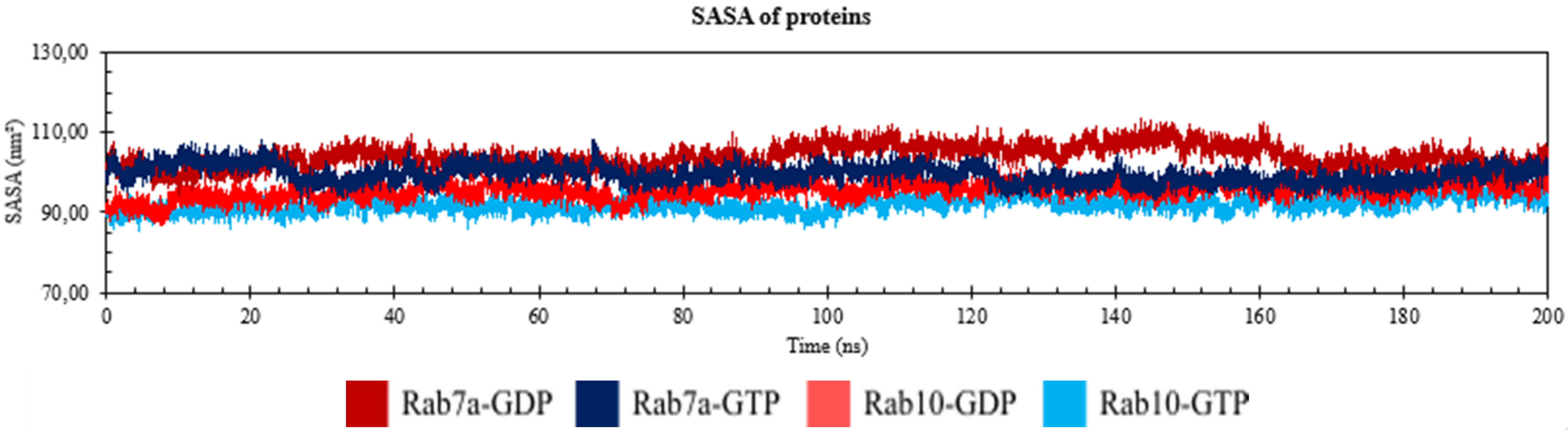


RESULTS AND DISCUSSION

Molecular dynamics study

- **SASA analysis:** We can infer that the disordered movements of switch 1 and the absence of γ-phosphate in GDP contribute to the increase in SASA.

Figure 9. SASA of G-domain of Rab7a and Rab10.



CONCLUSIONS AND FUTURE WORK

Molecular dynamics study

- MD simulations used in this study were able to obtain **notable differences in the switch 1** region of Rab's, enabling the identification of its active and inactive states.

- We hypothesized that the flexibility of the switch 1 sensitive region can be used as an indicator of in silico studies that search potential competitive inhibitors based on nucleotides against these targets;

- The next steps of this research is to extend the analyzes to evaluate the on-to-off process of 44 representatives of human Rab GTPases.

Thank you for your attention!

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Thanks

CNPq CAPES FAEPA HCFMRP STI