1.1	**Proposal**	Master thesis project
1.2	**Name**	Molecular Dynamics simulations of guanine nucleotides binding affinity to GTPases RhoA and RhoB
1.3	**Description**	RhoGTPases act as molecular switches by cycling between inactive (GDP-bound) and active (GTP-bound) states to regulate cellular functions like migration and morphology [1, 2]. In turn, RhoGTPases are regulated by three protein classes: *(1)* Guanine nucleotide Exchange Factors (GEFs) trigger RhoGTPases activation by catalyzing GDP-GTP exchange; *(2)* GTPase-Activating Proteins (GAPs) regulate inactivation by promoting GTP hydrolysis; *(3)* Guanine nucleotide Dissociation Inhibitors (GDIs) sequester proteins in cytosol, preventing membrane localization [3]. RhoA and RhoB are part of the RhoGTPase (sub-)family. Despite sharing remarkable structural homology ($\sim 85\%$), they exhibit distinct regulatory functions due to sequence variations in their functional domains. These structural differences affect their interactions with GAPs, GEFs, GDIs, downstream effector proteins[1, 2], leading to the main research question of this project: How do these two highly similar proteins achieve such different functionalities? **Objectives:** **• Structural and Dynamical Characterization** Perform *all-atom* Molecular Dynamics (MD) simulations of RhoA and RhoB in complex with guanine nucleotides (GDP and GTP) using experimentally determined structures. This analysis will elucidate the structural conformations and dynamical behaviors that distinguish these two closely related GTPases. **• Comparative Binding Affinity Assessment** Perform enhanced sampling MD simulations to extract GDP and GTP from their respective binding pockets in RhoA and RhoB, enabling quantitative comparison of the binding affinities, i.e., free energies, between these two GTPases for their guanine nucleotide substrates. This represents a potential first direct computational investigation addressing the kinetic differences in GDP release and GTP binding between these proteins. Optionally, artificial intelligence techniques can be used to identify key mechanistic differences between RhoA and RhoB and to increase sampling efficiency. **References:** [1] Antje Schaefer, Nathalie R Reinhard, and Peter L Hordijk. "Toward understanding RhoGTPase specificity: structure, function and local activation". In: *Small GTPases* 5.2 (2014), e968004. [2] AJ Ridley. "RhoA, RhoB and RhoC have different roles in cancer cell migration". In: *Journal of microscopy* 251.3 (2013), pp. 242–249. [3] Jacqueline Cherfils and Mahel Zeghouf. "Regulation of small GTPases by GEFs, GAPs, and GDIs". In: *Physiological reviews* 93.1 (2013), pp. 269–309.
1.4	**Work environment**	The student will take part in a cooperative project between the Computational Science Lab and the Computational Soft Matter Lab at the University of Amsterdam. These groups include experts in modeling of biological systems, at cellular and atomic level. By means of MD simulations, the student will be able to delve into the complexity of biological problems, contributing to a broader research focused on the role of RhoGTPases RhoA and RhoB in endothelial integrity.
1.5	**Expectations**	The student should have previous expertise with MD simulations and/or computational modeling of biological systems at cellular level.
1.8	**Research Tags**	MD simulations, Enhanced Sampling, Computational Biology, RhoGTPases
1.9	**Programmes**	Computational Science (joint degree UvA/VU), Chemistry (track–joint degree UvA/VU), Physics and Astronomy: Biophysics and Biophotonics (track–joint degree UvA/VU)
1.10	**Contact**	A. Acquaviva a.acquaviva@uva.nl, J.M. Hoffmann j.m.hoffmann@uva.nl