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## Fragment-based Discovery Aiming at a Novel Modulation of Malate Dehydrogenase and Beyond

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DOI:  
[10.33612/diss.150386440](https://doi.org/10.33612/diss.150386440)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2021

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*  
Reyes Romero, A. (2021). *Fragment-based Discovery Aiming at a Novel Modulation of Malate Dehydrogenase and Beyond*. University of Groningen. <https://doi.org/10.33612/diss.150386440>

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## Appendix

## SCIENTIFIC CONTRIBUTIONS

### List of publications

**The (R)-Enantiomer of the 6-Chromanol Derivate SUL-121 Improves Renal Graft Perfusion via Antagonism of the A1-Adrenoceptor.** Nakladal D, Buikema H, **Romero AR**, Lambooy SPH, Bouma J, Krenning G, Vogelaar P, van der Graaf AC, Groves MR, Kyselovic J et al.; *Sci. Rep.* 2019, 9 (1), 13; DOI:10.1038/s41598-018-36788-0

**Production of “Biobetter” Variants of Glucarpidase with Enhanced Enzyme Activity.** Al-Qahtani AD, Bashraheel SS, Rashidi FB, O’Connor CD, **Romero AR**, Domling A, Goda SK; *Biomed. Pharmacother.* 2019, 112, 108725; DOI:10.1016/j.biopha.2019.108725

**Oligomeric protein interference validates druggability of aspartate interconversion in *Plasmodium falciparum*.** Batista FA, Bosch SS, Butzloff S, Lunev S, Meissner KA, Linzke M, **Romero AR**, Wang C, Müller IB, Dömling ASS et al.; *Microbiology Open* 2019, e779; DOI:10.1002/mbo3.779

**Rapid Approach to Complex Boronic Acids.** Neochoritis CG, Shaabani S, Ahmadianmoghaddam M, Zarganes-Tzitzikas T, Gao L, Novotná M, Mitríková T, **Romero AR**, Irianti MI, Xu R et al.; *Sci. Adv.* 2019, 5 (7), eaaw4607; DOI:10.1126/sciadv.aaw4607

**Oligomeric interfaces as a tool in drug discovery: Specific interference with activity of malate dehydrogenase of *Plasmodium Falciparum* *in vitro*;** Lunev S, Butzloff S, **Romero AR**, Linzke M, Batista FA, Meissner KA, Müller IB, Adawy A, Wrenger C and Groves M; *PLoS One*, 2018; 13(4): e0195011. DOI: 10.1371/journal.pone.0195011

**Synthesis and Enantiomeric Separation of a Novel Spiroketal Derivative: A Potent Human Telomerase Inhibitor with High *in Vitro* Anticancer Activity;** Fuggetta MF, de Mico A, Cottarelli A, Morelli F, Zonfrillo M, Ulgher F, Peluso P, Mannu A, Deligia F, Marchetti M, Roviello G, **Reyes Romero A**, Dömling A and Spanu P; *J. Med. Chem.*, 2016, 59 (19), pp 9140–9149, DOI: 10.1021/acs.jmedchem.6b01046

**Gliptin Repurposing for COVID-19;** Ruiz Moreno AJ, **Romero RA**, Neochoritis C, Velasco-Velázquez M, Groves MR, Domling A; *ChemRxiv.*, 2020 <https://doi.org/10.26434/chemrxiv.12110760.v1>.

**Benchmark of Generic Shapes for Macrocycles; Romero RA\***, Ruiz Moreno AJ\*, Velasco-Velázquez M, Groves MR, Domling A; *J. Chem. Inf. Model.* (2020) doi:10.1021/acs.jcim.0c01038

**STXBP6, reciprocally regulated with autophagy, reduces triple negative breast cancer aggressiveness;** Lenka G, Shan J, Halabi N, Abuaqel S, Goswami N, Schmidt F, Zaghlood S, **Romero RA**, Subramanian M, Boujassoum S, Al-Bozom I, Gehani S, Al Khori N, Bedognetti D, Suhre K, Ma S, Dömling A, Rafii A, and Chouchane L; *Clin. Transl. Med.* 10, (2020)

**Discovery of covalent phosphatase inhibitors across six synthetic mass scales;** Sutanto\*, Shaabani S\*, **Romero RA\***, Hadian M, Holak TA, Groves RM, Dömling A; submitted.

**A Fragment-Based Approach Identifies an Allosteric Pocket that impacts Malate Dehydrogenase Activity;** **Romero RA**, Lunev S, Popowicz GM, Calderone V, Gentili M, Sattler M, Plewka J, Taube M, Kozak M, Holak TA, Dömling A, MR Groves; submitted.

### List of posters

**Allosteric Inhibition *in Vitro* of *Plasmodium Falciparum* Malate Dehydrogenase by Small Molecule and its Proposed Mechanism of Action.** **Romero AR**, Popowicz G, Groves M, Sattler M, Dömling A; *New Frontiers in Structure-Based Drug Discovery*; Sep23 – 25, 2019, Florence, Italy

**Fragment Based Design to Disrupt the Oligomeric Interface of *Plasmodium Falciparum* Malate Dehydrogenase.** **Romero AR**, Lunev S, Popowicz P, Calderone V, Gentili M, Plewka J, Kozak M, Sattler M, Dömling ASS and Groves MR; *Helmholtz Training Course on Integrative Structural Biology*, Oct 22 – 26, 2018 at CSSB, Hamburg, Germany

**A New Toolkit in Drug Target Validation: the Protein Interference Assay.** **Romero AR**, Batista FA, Bosch S, Lunev S, Linzke M, Groves M and Wrenger C; *HDDC 2018*; April 26/27; Munich, Germany

**How to Design Specific Molecules Interacting with Aspartate Aminotransferase of *P. Falciparum*.** **Romero AR**, Groves MR and Dömling AS; *EUROPIN Summer School on Drug Design*, September 17 – 22, 2017; Vienna, Austria

### **List of presentations**

Flash talk, *New frontiers in structure-based drug discovery*. 23 – 25 September 2019, Italy, Florence.

## **Secondments**

Helmholtz Zentrum München (six months, supervisors: Prof. Michael Sattler, Dr. Grzegorz Popowicz), Giotto Biotech (three months, supervisor: Dr. Matteo Gentili), Jagiellonian University (twelve months, supervisor: Prof. Tad Holak).

## Chapter contributions

In **Chapter 2** I have recombinantly expressed the proteins, performed the high throughput screening with NMR followed by MST validation and TSA. Next, I tested the biological activity and crystalized the fragment. Finally, I analyzed the results and wrote the final version of the manuscript.

In **Chapter 3** I conceptualized the automatization of conformers generation of macrocycles with Moloc by keeping constant correspondence with the developer of the software. Next, I performed the computational work, analyzed the data and wrote the manuscript.

In **Chapter 4** I have recombinantly expressed the protein, performed the reproduced the conditions of crystallization. In addition, I coordinated the collaboration with the mass spectrometry laboratory to determine the mechanism of action. Next, I performed the initial activity assays to identify the inhibitors, check the reproducibility. Finally, I performed the computational analysis, wrote the manuscript and analyzed the results.

In **Chapter 5** I did the docking with CovDock, analyzed the results, wrote the final version of the manuscript.

## Acknowledgments

Finally, the end! It has been a long journey since I decided to undertake this important career path within the Marie Curie European project, AEGIS. During these years of my PhD I have met many people who had a strong impact on both my professional and personal growth. Hence, I would like to thank them all. I already apologize in advance if I omit someone.

To begin with, I would like to thank all the staff of the Department of Drug Design and, particularly, Prof. Alexander Dömling and Prof. Matthew Groves for supervising my work and hosting me in their laboratory.

I would like to remind you, Alex, as mage Merlin who encourages Arthur to always go further towards the limits of the unknown “chemical space”. Doing science with you has been a fundamental moment of growth in order to learn to go beyond our personal limits, while keeping on improving ourselves. You have been a great mentor for me and I learned a lot from you, especially the discipline and the perseverance on each single project you have proposed me, allowing me the freedom of developing them as best as I could. Another thing that I will never forget were the moments of hilarity to alleviate the hard work of scientific reasoning. I would summarise them in one sentence: “Edamus, bibamus, gaudeamus: mens sana in corpore insano!”.

Matthew, thank you for introducing me to structural biology and protein crystallography. I remember the first day I joined the department and you welcomed me with a “Welcome to the cold!” and at the same time you introduced me to the things I was supposed to read and learn. At first, I was unsure whether I would be able to learn to purify my first protein myself! But now I am very happy to possess this new background of knowledge: thanks a lot for that. In this sense, I agree with Palpatine: “Anakin, if you really want to understand the great mystery, you have to investigate it in all its aspects, not only the dogmatic and narrow Jedi one”. If so, well, then I took the first step towards an understanding not only from a chemical but also biological point of view of drug design. I feel very honored to have spent these four years sharing the research with you and Alex!

I can't find the words to thank you, Jolanda, for your continuous support in different administrative issues, coordinating the traceability of my parcels during my AEGIS secondments abroad. I will miss your kindness and patience in your explanations.

Next, I would like to acknowledge the members of the assessment committee, Prof. Carlos Camacho, Prof. Anna Salvati and Prof. Amalia Dolga for taking the time to read my thesis, evaluate it and send me valuable suggestions for improvement.

Dinos, it's been a real pleasure meeting you! Our collaboration in the boronic acid project resulted in a publication of high impact factor. I hope in the future to have the opportunity to work and meet you again, perhaps in Crete.

Next, I'd like to express my gratitude to my dear Mexican friend Angel. Angel, it was a pleasure to welcome you to my home, spending the Christmas together and introducing you to the gastronomy of Verona. Although we may have different points of view, you're one of the few I can truly call "collaborator". I hope that a pinch of Italian esprit will always follow you in your future travels and keep on working on some new ambitious chemoinformatics projects. Un abrazo y espero verte pronto para comer muchos tacos en compañía.

Serjey, Fernando, Ameena, Wenjia, Kai, Rick, Alaa, Chao, Juliana, Niels, Eswar, Marleen, Soraya, Juliana I send you all a warm hug and a good luck for your future career. I think I'm really going to miss the interculturality community and the nice atmosphere that you created around the offices where we used to have coffee together. A big thanks to Rick who helped me to check over the translation of the summary of the thesis and collaborate with me in the context of the phosphatase project. A warm hug to my friends Arianna, Paola, Marta, Francesca, Vincenzo and Annarita who contributed to make my PhD in Groningen a bit more "Italian".

When I arrived in Groningen, I didn't know anyone. Thank you very much Natalia, Edwin, Trifonas, Eman, Vishnu, Yuanze, Naveen, Ajay for making the environment comfortable and helping me fit in with the research group. Fandi, Shabnam, I was please to have collaborated with you. Thanks to you for choosing PTP1B as my model to test your library. I hope to see you all soon in some medical chemistry conference.

I would also like to thank my students for their perseverance and hard work, Marina Ika Irianti and Marina Rodriguez Rubio. I hope I have shared with you the passion in scientific research and congratulations for your recent achievements. Keep in touch and hope to see you again.

I am sincerely grateful to Prof. Michael Sattler and Dr. Grzegorz Popowicz for accepting me at the Helmholtz Zentrum München where I applied NMR in fragment screening for my AEGIS projects. It has been a very stimulating and unforgettable experience that allowed me to add new knowledge to my personal “toolbox”. Thanks very much Arie for your help in cloning the wild type MDH in the pETM vectors and for your patience when you waited for me and João before going to Tollwood festival: we were really in delay! I miss my stay in Munich and the friendly atmosphere in the laboratory and I’m happy to have met Eleni, André, Alisha and Ana. Astrid. Thank you, Astrid, for making the laboratory an efficient and productive workspace, welcoming me with a smile every morning.

And now you, my dear AEGIS friends and colleagues! Ave, Charlie, Engi, Francesca, Giulia Joy, João, Laura, Maxime, Markella, Patrick, Ryan, Roberto, Valeria. You have made my journey into the open sea of science unforgettable and you’ve been like a little family to me. I catch the occasion to thank Dr. Eva Schlosser for her valuable coordination work within the AEGIS consortium, organizing our annual meetings with great care and commitment. I would also like to thank the European community within the Marie Skłodowska-Curie Actions, Horizon 2020, for giving us this privilege to mature as young scientists by travelling in different institutions and opening our minds to different perspectives and points of view.

A sincere thanks for the CERM staff of Florence – Linda, Prof. Marco Fragai and Dr. Vito Calderone. Thanks to your support and collaboration I was able to get the data I needed to write the second chapter of my thesis. I also want to thank Alessandro for looking over me during the first days in Florence and sharing his workbench with me. A big hug also to Mercia, Sabrina, Domenico, Alessio, Veronica, Sara, Francesca, Lucia, Stefano, Giovanni, Dafne, Silvia and Josè. A special thanks to Tommaso Martelli, Stefano Cardelli and Laura Bassani for helping me to settle into my new workplace during the first weeks at Giotto biotech.

Furthermore, I would like to thank Prof. Tad Holak for giving me the opportunity to do the activity experiments in your laboratory in Krakow for the PTP1B project. I take this opportunity to send a strong hug to all you– Radek who now loves airplane journeys (!), Mira, Jacek, Monika, Aneta, Beata, Kasia, Bogdan, Ewa, Łukasz, Dominik, Magda, my former officemate Przemek, Justyna (Atilko!). Thank you from the bottom of my heart for getting me closer to Polish culture with love: I appreciate it a lot. Bardzo za wami tęsknię! A hug to my playmates, Luois, Jack, Ismael, Julian, Alex Matsuda. I look forward to come back to Krakow and play pool with you again. Same place, same time!

I also thank the x-ray platform and the mass spectrometry centre, in particular Piotrek, Przemek and Urszula. Special greetings also to Ela, I think the most brilliant and passionate crystallographer I have ever met on my (limited) travels in Europe. I'll never forget our trip to BESSY and the Korean dinner: it was delicious. Keep it up!

I catch the occasion to thank Prof. Grzegorz Dubin for letting me use the instruments in its laboratory and perform my experiments. I felt welcomed by your students who I am still in contact too! Also, the school in Zakopane last year has been a nice experience for me.

A big hug to Frank, Mariella, Federico and Niccolò. You have been and still are very important friends for me. Last but not least, I want to thank my family for always being on my side, listening and supporting me.

Ringrazio mia moglie, Maria Chiara, per essere stata sempre al mio fianco. Credo che non ci siano parole per dirti quanto tu sia importante per me.

*Atilio*

11<sup>th</sup> January 2021