INTERNSHIP OPPORTUNITY
Massachusetts Institute of Technology / Whitehead Institute, MA, USA (2021-2022)

Laboratory of Professor David M. Sabatini
Supervisor: Dr. Kacper B. Rogala, Postdoctoral Fellow
http://sabatinilab.wi.mit.edu

Prospective students, please contact Dr. Rogala directly via email: rogala@wi.mit.edu. Attach your CV, and write a few lines (under a page long) explaining:
(1) why this internship opportunity is interesting to you, and
(2) why you think you will be the right candidate to carry out this project.
The most promising candidates who demonstrate a great interest in this project will be invited for an online interview.

PROJECT TITLE: Structural Biology and Drug Discovery.
Trapping the scavenged and recycled nutrients of pancreatic cancer cells

Figure 1. Proposed therapy against proliferation of pancreatic cancers that scavenge and recycle nutrients. (A) Macromolecules, including albumin, enter the lysosomes via the macropinocytosis and autophagy pathways, and are further degraded into free amino acids. SLC38A9 senses and releases digested amino acids to cytosol, effectively fueling growth and proliferation of those cancer cells. (B) Even though macropinocytosis and autophagy pathways still deliver cargo to lysosomes for degradation, the resulting amino acids will become trapped inside the lysosomes due to (B-1) small-molecule inhibition of the SLC38A9-driven efflux, or (B-2) due to targeted degradation of SLC38A9.

SUMMARY OF SCIENCE: Aggressive pancreatic cancer cells tend to quickly grow out of control and crowd out normal cells. Once spread to neighboring organs, they grow out again to form new tumors that often damage those organs. These aggressive cancer cells are able to escape cytotoxic chemotherapy treatments and survive in near-starvation conditions. So what
is it that makes them so hard to kill? The answer to that question lies in the fact that these cancer cells have completely reprogrammed their metabolism. Instead of waiting for blood vessels to deliver essential nutrients for growth, these cancers actively scavenge their environment for extra food, and they also recycle their own cellular components at high rates. These two processes of scavenging and recycling deliver large macromolecules (e.g. protein and nucleic acids) into specialized destruction factories of the cell, known as lysosomes. Inside the lysosomes, and under very low pH conditions, those macromolecules are broken down into individual building blocks, such as amino and nucleotides, which in turn fuel cancer growth and expansion.

A NOVEL THERAPEUTIC IDEA. To fight aggressive pancreatic cancers that rewire their metabolism, many scientists are currently working out ways of blocking their ability to scavenge nutrients. But, what if, instead of preventing cancer cells from eating, we simply locked the fridge? Imagine a situation where cancer cells are no longer able to open lysosomes and use this digested food in the first place. After having their main food supply cut off, the scavenging-addicted cancer cells will stop growing. At the same time, the normal cells would ultimately not care, because their food and energy do not come from scavenging. The Sabatini Lab of MIT has recently discovered a molecular machine - a membrane protein called SLC38A9, that acts as a gatekeeper for releasing nutrients from lysosomes to cytosol. Our preliminary data suggest that if SLC38A9 is deleted in cancer cells, the lysosomes never open again, and all the food is trapped inside – unable to fuel cancer growth. See Fig. 1 for a conceptual picture of this idea, and our recent papers listed in the last section of this document.

THE AIM OF THIS PROJECT. This exciting finding mentioned above led us to pursue a project, in which we aim to learn more about how the SLC38A9 food gate works. Particularly, we want to understand what the gate looks like on the atomic level, and how the gating process is regulated. We will use structural tools, such as electron cryomicroscopy (cryo-EM) and X-ray crystallography, to build an image of human SLC38A9 in complex with the mTOR machinery, and specifically -- during the process of amino-acid gating - to and from lysosomes. These detailed images will help us and other researchers develop drugs that can specifically block SLC38A9’s gating function, thus cutting trapping all the scavenged and recycled nutrients inside of lysosomes. This is a novel treatment idea, and we expect that it will starve aggressive tumors whilst sparing all other normal cells that do not scavenge proteins. Thus, a large part of this project will be also dedicated to performing early drug discovery experiments, where we will use large libraries of chemical compounds to screen for molecules that bind to SLC38A9 and interfere with its nutrient transport function. Our aim is to identify and validate lead molecules that can be later advanced to the clinic. Particularly, we will take a two pronged-approach to develop direct inhibitors of SLC38A9, but also specific bifunctional molecules that target SLC38A9 for proteasomal degradation (see Fig. 1B).

LONG-TERM PERSPECTIVE. In this project, we want to provide a better approach for a cure via a novel mechanism, so that in 10 years patients diagnosed with pancreatic or other forms of aggressive cancer will not perish within months, but have a real chance at surviving this terrible disease. We aim to create a next-generation drug that can be administered immediately after diagnosis, so that it completely halts the growth of metabolically-rewired aggressive cancer cells whilst minimizing any potential side effects in healthy tissues. Such
drugs will give precious time to oncologists, so that they can thoroughly investigate the vulnerabilities of cancer at hand, and then choose the best combination therapy in order to kill affected cells.

**SUMMARY OF TRAINING:** This is an exciting opportunity to work in one of the best American biology labs, and in one of the top research institutes in the world – MIT / Whitehead Institute. You will have a chance to be a part of a team of very driven and dedicated scientists whose ambition is to help find a cure against devastating aggressive cancers, such as pancreatic.

**WHAT WILL YOU LEARN?** In your internship, you will work directly side-by-side with your supervisor, Dr. Kacper Rogala, and receive training in protein chemistry, and also in many specialized approaches aimed at understanding protein structure and function. You will also work towards establishing a screening and validation platforms for drug discovery efforts against aggressive pancreatic cancers. At the end of your training you will have gained substantial hands-on experience in working with proteins and have a good grasp of the latest cutting-edge technology to study them. You will also learn how to perform various *in vitro* and *in cell* assays to study the effects of small molecule drugs on proteins and cells. Most importantly, this project will give you the necessary exposure and skills required for a successful PhD study or a placement in a pharmaceutical or a biotechnology company.

Moreover, through their passion and grit, most of our Master’s students have significantly contributed to many research stories that came out from our lab, which deservedly earned them co-authorship on major publications. Importantly, all of our trainees to date have truly spread their wings in our lab, and have gone to pursue PhD degrees or careers in the pharma/biotech sector.

**SPECIFIC SKILLS THAT YOU WILL LEARN AND GET EXPOSED TO IN YOUR INTERNSHIP**

**Protein chemistry / structural biology / biophysics:**
- Recombinant expression of proteins and protein complexes in bacteria, insect and mammalian cells
- Protein purification using a range or chromatographic techniques
- Working with membrane proteins
- Development of conformation-specific nanobodies against target proteins
- Evaluation of protein function and protein-protein interactions with a range of biochemical and biophysical techniques: functional assays, ITC, FP, CD, mass photometry, SEC-MALS, DSF and many more
- Cryo-electron microscopy – sample preparation and imaging
- X-ray crystallography – crystallization, crystal mounting, data collection and structure solution
- Data analysis and computational evaluation of protein structures.

**Early drug discovery:**
- Sterile tissue culture techniques
- Design and execution of high-throughput drug screening experiments
- Evaluating potential drug hits *in vitro* and *in cells* with various specific assays and biophysical instruments, such as:
  - liposome-based transport assays
WHO ARE WE LOOKING FOR? This project will be most suitable to a bright, highly motivated and dedicated Master’s student with keen interest in protein biochemistry, structural biology and drug discovery. Modern science is a team sport so good communication skills are key. You are expected to be a team player with an excellent command of English. You are also expected to have some prior wet-lab experience. In other words, we look for smart, enthusiastic, dedicated, meticulous and well-organized students with a background in biology or chemistry (for example: biochemistry, molecular biology, biophysics, biotechnology, bioengineering, chemical biology, pharmacology, chemical engineering or similar). If you are thrilled by the prospect of discovering fundamental biological mechanisms, and are prepared to dedicate your time to exploit these mechanisms in our shared quest to fight cancer, then this project is for you.

WHEN CAN YOU START AND HOW LONG CAN YOU STAY? We are quite flexible in terms of your start date, and can take you in as early as June 2021. The earlier the better, but it is not so critical -- if we like you, we will be happy for you to start later in the summer or fall if that’s better aligned with your university courses. More importantly, we expect from you a minimum of a 6-months commitment towards this project. It will be an advantage if you can stay with us for longer, and we would encourage you to do so.

WHAT ABOUT FUNDING? We will provide you with bench space, lab consumables, supervision and your visa documentation. We cannot, however, cover any living expenses, and therefore it is very important that you put an effort into obtaining funding for your internship from your home university or your home country. We are ready to work with you to craft a compelling research proposal that you can submit for competitive scholarships. There are usually a number of funding schemes that one can apply for, and our students have had good success in securing those in the past. Please get in touch, and together we will devise the best strategy that works for all of us.

DO WE OFFER ANY OTHER PROJECTS? Yes, we do. SLC38A9 is our top priority project at the moment, and we put the most effort into it. However, we also actively pursue a number of other mTOR-related projects, and many of our students end up following those directions. Please get in touch if you would like to hear more about these projects!

RELEVANT ARTICLES FOR FURTHER READING:


• Sabatini (2017) *PNAS* 114(45):11818-11825. PMID: 29078414 – an accessible overview of the mTOR pathway and the history of discovery in the field.