INTERNSHIP OPPORTUNITY
Massachusetts Institute of Technology / Whitehead Institute, MA, USA (2020)

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
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, Biochemistry and Drug Discovery.
Cutting off the amino-acid supply lines of Ras-transformed aggressive cancer cells

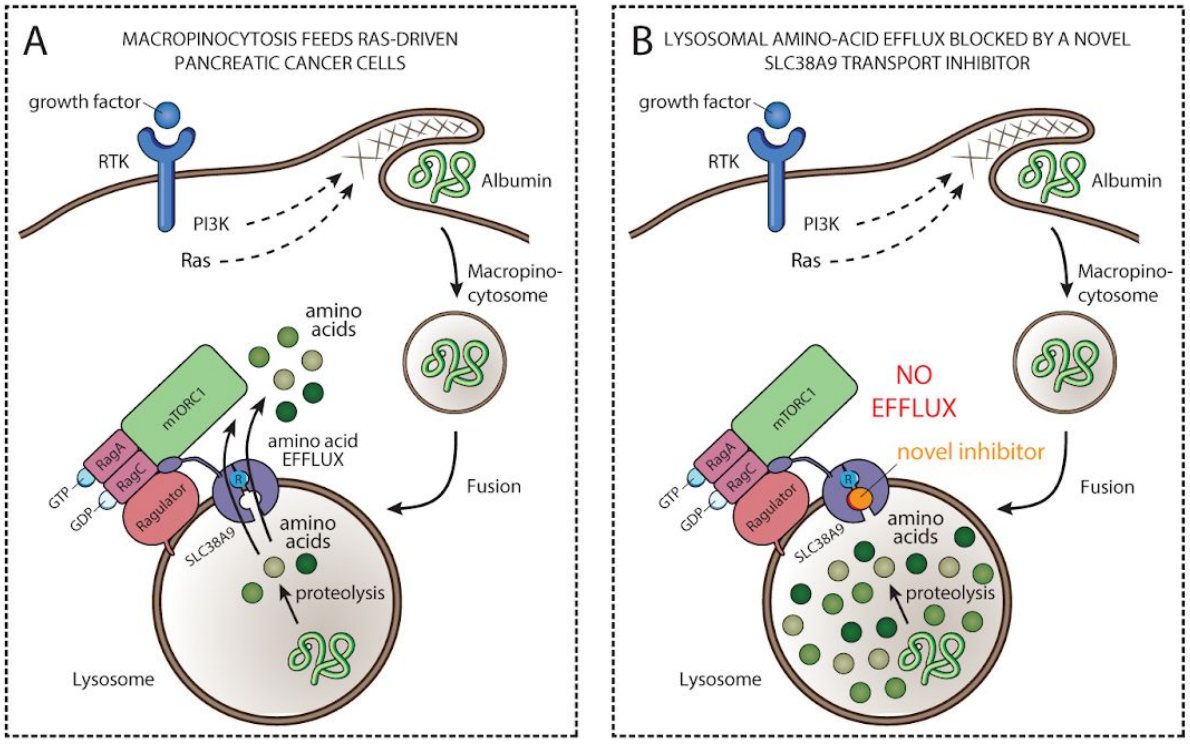

Figure 1: Proposed therapy against Ras-transformed aggressive cancer cells. *(**A**) Albumin is internalized via macro-pinocytosis, and degraded in lysosomes. The resulting free amino acids are sensed by SLC38A9 and released to cytosol in an arginine-dependent manner to fuel cancer cell growth and proliferation. (**B**) A specific inhibitor of SLC38A9 will trap digested amino acids inside lysosomes, halting growth of cancer cells.*

SUMMARY OF SCIENCE: Aggressive 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 Ras-transformed cancers achieve such high growth rates through active scavenging of nutrients from their environment. An important class of nutrient is protein, and aggressive tumors specifically rely on scavenged

protein for their growth and expansion. These tumors digest and recycle scavenged proteins to generate more energy, and to produce glucose, fat or other proteins. Digested proteins are stored in small compartments called lysosomes, which act as ready-to-go bags of food that can be used as fuel for cancer growth and expansion.

A NOVEL THERAPEUTIC IDEA. To fight such aggressive cancers, many scientists are currently working out ways of blocking their ability to scavenge proteins. But, what if, instead of preventing cancer cells from eating, we simply lock the fridge? Imagine a situation where aggressive tumors 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 protein-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 Laboratory of MIT has recently discovered a molecular machine - a protein called SLC38A9, that acts as a gatekeeper for releasing food from the lysosomal bags. Our preliminary data suggest that if SLC38A9 is removed from 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 AIMS 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 **X-ray crystallography** and **cryo-electron microscopy**, 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 images will help us and other researchers develop drugs that can specifically block SLC38A9's gating function, thus cutting off the food supply lines to aggressive cancer cells. 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 a large library of chemical compounds to screen for molecules that affect the function of SLC38A9 and its regulators. Our aim is to identify lead molecules that can be later advanced to the clinic.

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 Ras-transformed aggressive cancer cells whilst minimizing any potential side effects in healthy tissues. Such drug 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, lung and colon.

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 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.

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 conformation-specific nanobodies against target proteins
- Biochemical and biophysical evaluation of proteins and their complexes with: CD, SEC-MALS, ITC, FP, 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.

Pre-clinical 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
    - differential scanning fluorimetry
    - metabolite profiling with LC-MS
    - biolayer interferometry
    - surface plasmon resonance

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, keen, dedicated and meticulous students with a background in any of the following areas (or similar): biochemistry, structural biology, molecular biology, biophysics, biotechnology, chemistry, chemical biology or pharmacology. 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 March 2020. 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.

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 you should aim to obtain funding for this 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 is usually a number of funding schemes that one can apply for. 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, and we put the most effort into it. However, we also have funding for other mTOR-related projects, so please get in touch if you would like to hear more about them.

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.*
- Wang *et al.* (2015) Science 347(6218):188-94. PMID: 25567906 – *discovery of SLC38A9's involvement in the mTORC1 pathway.*
- Wyant *et al.* (2017) Cell 171(3):642-654.e12. PMID: 29053970 – *discovery of SLC38A9's role in effluxing amino-acids from lysosomes to cytoplasm, and the implications for inhibition of growth of Ras-transformed aggressive tumors.*
- Renaud et al. (2018) Nat Rev Drug Discov. 17(7):471-492. PMID: 29880918 – *a good overview of the applications of cryo-EM in drug discovery.*