Stanford MEDICINE | Rogala Laboratory

INTERNSHIP OPPORTUNITY (2022-2023)

Stanford University, USA

Laboratory of Dr. Kacper Rogala

From protein mechanisms to targeted therapeutics

We are accepting Master's students for a thesis research project in 2022-2023.

We are a brand new lab at Stanford, headed by Dr. Kacper Rogala, and our team will be transitioning from MIT to Stanford in the spring of 2022. We are very excited for this new chapter, and we cannot wait to get started.

Prospective students, please contact Dr. Kacper Rogala directly via email: rogala@stanford.edu.

When contacting Kacper, please provide the following:

1. Write a few paragraphs about yourself — what drives you, why you found our research interesting, and what you are looking to accomplish in your Master's thesis lab.
2. Your academic CV.
3. A reference letter (or a contact to a reference writer).

We will be very happy to tell you more about potential projects, our style of doing research, and importantly — how this lab can be an opportunity for you grow scientifically, and how in this lab, the translational nature of our work can make an impact in patients' lives — by cracking challenging basic science questions and developing novel therapeutics.

RESEARCH MISSION | The survival of every organism requires **biochemical sensing** and **trafficking of nutrients** to appropriately regulate metabolism and many fundamental aspects of physiology.

How are nutrients recognized by their sensors? How is their transport across cellular and intracellular membranes regulated? And, how is nutrient sensing integrated with other chemical signals, such as hormones, to determine cellular decisions, especially the decision: **to grow** or **not to grow**?

We are a team of highly driven and dedicated scientists, working at the interface of **biology** and **chemistry** to answer these fundamental questions at the level of atoms and single molecules. We use a full range of approaches from **structural biology**, **chemical biology**, **cell biology**, **biophysics**, and **biochemistry** — to discover new basic knowledge, and contribute to the development of therapeutics for devastating diseases of growth, including **cancer** and **tuberous sclerosis**.

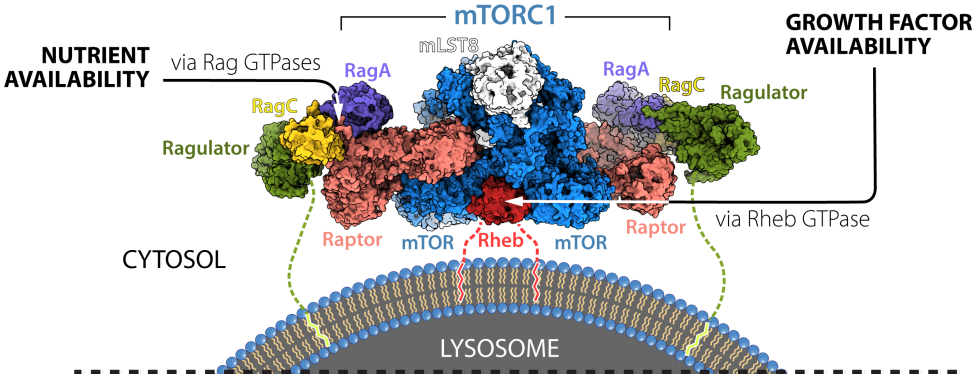


Figure 1. mTORC1 is a multisubunit protein kinase which balances cellular growth and recycling. Our lab elucidated the structural mechanism by which mTORC1 reads the availability of amino acids whilst docking on the lysosomal membrane (Rogala *et al.* (2019) PMID: 31601708).

ABOUT THE ROGALA LAB | We are a new lab at Stanford, located in the Biomedical Innovations Building. Our lab is a part of the **Stanford Cancer Institute**, and two basic-science departments in the School of Medicine: **Structural Biology**, and **Chemical and System Biology**. We are captivated by basic biological questions of how cells sense and traffic nutrients inside cells, and we use that newly discovered knowledge to develop experimental drugs which in the long-term will help patients whose cellular growth mechanisms are dysregulated by diseases. The work that we do is highly collaborative, and we run many projects together with other labs who share our mission, and who complement our approaches.

ABOUT THE PI | **Dr. Kacper Rogala** is an Assistant Professor in the Department of Structural Biology and the Department of Chemical and Systems Biology at the Stanford University School of Medicine. He is also a Member of the Stanford Cancer Institute.

Kacper was born and raised in Poland, and educated in three wonderful British cities: Oxford, London and Edinburgh, where he studied chemistry of living things, or simply — biochemistry. During his studies, Kacper developed a deep passion for proteins — how they work, what they look like, and how they interact with other proteins and small molecules. This passion led him to pursue a trans-Atlantic postdoc between two Cambridges: one in the UK and one in Massachusetts. As a researcher at MIT, the Whitehead Institute, the Broad Institute, and the MRC Laboratory of Molecular Biology, Kacper began unraveling the mechanisms of nutrient sensing on the surface of lysosomes.

Now as a principal investigator at Stanford, Kacper and his team are leading the charge towards detailed understanding of how proteins sense and traffic nutrients inside cells, and how the activity of these proteins can be modulated with chemical probes — for the benefit of patients.

You can read a short interview with Kacper here (from his time at MIT).

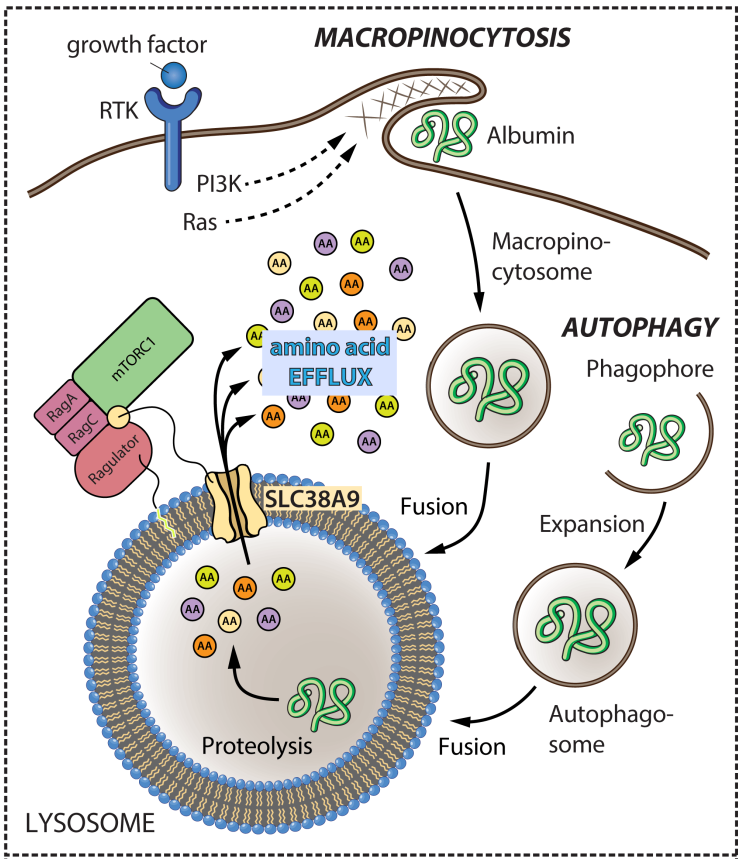
MASTER'S THESIS IN THE ROGALA LAB — WHAT EXACTLY DO WE STUDY? | We have two major research branches in our lab: (1) basic, and (2) applied. In the basic branch, we try to figure out how life works, and then we use that knowledge in the applied branch — to develop experimental drugs for the benefit of patients.

GROWTH DECISIONS. Our mission is to elucidate how cells make growth decisions — *to grow* or *not to grow* — based on their environmental conditions, such as availability of nutrients or growth factors. We make proteins responsible for these decision-making processes, and we try to figure out what these proteins look like and how they function. It turns out that many of these proteins interact with the surface of the lysosome, which beyond its nutrient-recycling function, also works as a sophisticated signaling center, able to sense the availability of nutrients and direct cellular metabolism. Working together, these proteins are able to adjust biological metabolism by switching between anabolism (growth) and catabolism (recycling) — in response to the environment, and in the matter of minutes. Collectively, we call these proteins "**the mTOR pathway**", because the major enforcer of these decisions is a protein kinase called mTOR (see our cryo-EM structure of mTOR elements in **Fig. 1** above).

For a fascinating story about mTOR, the wonder drug rapamycin, and the incredible impact that one strong-willed scientist can have on the world, check out this episode of Radiolab.

NUTRIENT TRAFFICKING. Beyond mTOR, we are fascinated by how nutrients are trafficked inside cells — by membrane protein transporters, and how that trafficking is hijacked and exploited to fuel cancer growth. For example, many RAS-transformed pancreatic cancers often elude even the harshest chemotherapy treatments, because they can survive in nutrient-poor environments while other cells cannot. These cancer cells metamorph themselves into macrophage-style consumers of extracellular protein. They internalize that protein, digest it inside lysosomes, and release it as amino acids to fuel their growth. It turns out that the release of digested amino acids from the lysosome to cytosol is fully controlled by nutrient transporters, and if we knock these transporters out, cancer cells no longer have access to that pool of digested food and starve to death (see **Fig. 2** for a schematic of how we are targeting one such transporter — SLC38A9).

This is just one example of how nutrient transporters can be exploited to specifically target cancer cells based on their unorthodox eating habits. Also, it turns out that beyond their canonical function of ferrying nutrients, these transporters also serve as receptors and regulators to actively fine-tune the cellular responses to fluctuating



nutrient levels. This is a completely uncharted territory, and with structural and chemical biology tools, our lab is spearheading efforts towards revealing how these **transceptors** (transporters + receptors) work and how they communicate with the growth machinery of the cell.

Figure 2. RAS-transformed pancreatic cancer cells recycle protein via macropinocytosis and autophagy to recover amino acids. The SLC38A9 transceptor communicates the availability of lysosomally-digested amino acids to mTORC1 and effluxes them into the cytosol. Our lab studies the gating mechanism of SLC38A9 and we work on developing it as a novel drug target against cancer cells that rely on it for survival in nutrient-poor conditions.

At all stages of our work, we develop binders — and by binders we mean small molecules or biologics (antibodies / nanobodies) that interact with a target protein. Such interactions often result in either inhibition or augmentation of cellular function of these proteins. And we use these binders as research tools, and also as starting points for the development of novel cancer therapeutics — in collaborations with chemists, computational biologists, bioengineers and clinicians.

MASTER'S THESIS IN THE ROGALA LAB — WHAT WILL YOU LEARN? | We are a team of highly driven and dedicated scientists whose ambition is to help find a cure against devastating diseases. Because we are a small lab, in your thesis project you will work directly side-by-side with your mentor, Dr. Kacper Rogala, and receive training in protein chemistry of large macromolecules and membrane transporters. Depending on your specific project, you will also have an opportunity to work towards establishing a screening and validation platform 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 future PhD study or a placement in a pharmaceutical or a biotechnology company.** 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 (OR GET EXPOSED TO) IN YOUR INTERNSHIP

<u>Protein chemistry / structural biology / biophysics:</u>

- 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,BLI, 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.

<u>Cell biology:</u>

- Sterile tissue culture techniques

- Pull-down assays
- Cell signaling experiments
- Isolation of organelles, and profiling with Western blotting and LC-MS
- Fluorescence microscopy — protein localization, FRAP, FRET

Early drug discovery:

- 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
    - biolayer interferometry (BLI)
    - surface plasmon resonance (SPR)
    - differential scanning fluorimetry (DSF)
    - metabolite profiling with LC-MS.

WHO ARE WE LOOKING FOR? | We welcome students from a broad range of science and engineering backgrounds — including **biochemistry**, **cell biology**, **molecular biology**, **biophysics**, **biotechnology**, **bioengineering**, **chemistry**, **chemical engineering**, **pharmacology**, **bioinformatics**, or similar.

In general, our projects are most suitable to highly motivated Master's students with keen interest in protein chemistry, structural biology, chemical biology and drug discovery. If you are thrilled by the prospect of discovering fundamental biological mechanisms and applying that knowledge in our shared quest to fight cancer, then this lab is for you.

Modern science is a team sport, so good communication skills are key, and we expect you to play well with others. We have both wet-lab and computational projects, and we would expect you to have some prior experience (either in the wet or the "dry" lab) if you'd like to be a part of these.

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 2022. 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 year or even in 2023, if that's better aligned with your university courses. More importantly, we expect from you **a minimum of an 8-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 will also pay the fees of your enrollment at Stanford as a visiting student. 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. mTOR and SLC38A9 are our top priority projects at the moment, and we put the most effort into them. However, we also actively pursue a number of other projects, and we will be happy for you to follow these directions. Please get in touch if you would like to hear more about these!

FEATURED PUBLICATIONS FROM THE ROGALA LAB

- **Rogala** *et al.* (**2019**) Structural basis for the docking of mTORC1 on the lysosomal surface. ***Science***, 366(6464):468-475. PMID: 31601708.
    - Featured in the 2021 edition of the Lodish *et al.* Molecular Cell Biology textbook.
    - News on the Whitehead Institute and the MIT webpages.
    - Two independent F1000 recommendations.

    - Spotlight article in *Trends in Biochemical Sciences* by Jin Park, Gina Lee, and John Blenis.
    - Dispatch article in *Current Biology* by Wei Peng and Jenna Jewell.

- Shen and **Rogala** *et al*. (**2019**) Cryo-EM Structure of the Human FLCN-FNIP2-Rag-Ragulator Complex. ***Cell***, 179(6):1319-1329.e8. PMID: 31704029.
    - News on the Whitehead Institute webpages.
    - Dispatch article in *Current Biology* by Wei Peng and Jenna Jewell.

RELEVANT ARTICLES ABOUT SLC38A9 — FOR FURTHER READING:

- Wang *et al.* (**2015**) Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. ***Science***, 347(6218):188-94. PMID: 25567906.
  ⇒ Discovery of SLC38A9's involvement in the mTORC1 pathway.

- Wyant and Abu-Remaileh *et al.* (**2017**) mTORC1 activator SLC38A9 is required to efflux essential amino acids from lysosomes and use protein as a nutrient. ***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.