



# JOSHUA RABINOWITZ

LUDWIG PRINCETON

## The metabolic portraitist

By the mid-2000s, word had spread in certain circles that Joshua Rabinowitz was a man with a solution. Since starting his laboratory at Princeton University in 2004, he had pioneered a unique approach to the comprehensive measurement and analysis of metabolites—or metabolomics—and was applying his technologies to make important discoveries in yeast biology and virology. Scientists confronting metabolic puzzles in their studies were calling him with growing frequency. Among them was Craig Thompson, a pioneer of modern cancer metabolism research.

Thompson, then head of the University of Pennsylvania Cancer Center and now president of Memorial Sloan Kettering, paid a personal visit to Princeton to see Rabinowitz,

then a young assistant professor. After a tour of Rabinowitz's lab and technologies, Thompson pitched to Rabinowitz some of the problems in cancer research that he thought they might crack together. "A couple of minutes into Craig's talk, my brain started buzzing," says Rabinowitz. "I was sold on the intellectual challenge and life-improving potential of investigating these connections."

The meeting would draw Rabinowitz into a series of collaborations with cancer researchers, beginning with a pair of major studies that contributed to the development of a cancer therapy targeting the mutated metabolic enzyme IDH by the drug company Agios. A decade later, Rabinowitz's increasingly sophisticated exploration of the chemistry of life is



Photo by Flynn Larsen

transforming our understanding of systemic and tumor metabolism, opening exciting new possibilities for the prevention and treatment of cancer. On the strength of that research, Rabinowitz was in 2021 named founding director of a new Ludwig Branch based at Princeton University and dedicated to the study and disruption of cancer metabolism.

### **PANNING FOR NUGGETS**

A relatively cautious child, Rabinowitz enjoyed an idyllic upbringing in Chapel Hill, North Carolina, enjoying lazy days at the pool in summer and college basketball games (some featuring Michael Jordan) in winter. His parents, both political scientists, were pioneering quantitative methods for studying voter behavior. Home life was peppered with talk of both politics and mathematical models, and Rabinowitz showed an early talent for mathematics that he cultivated with the encouragement of his father.

In high school, beyond the standard challenges of being a teenager, Rabinowitz spent a year in Norway with his parents, who were there on sabbatical. After returning, his quest for a summer job landed him in a cancer research internship at nearby Duke University, getting his first taste of scientific research in the laboratory of William Peters, where he was charged with measuring cytokine levels in samples obtained from breast cancer patients. He stayed in Chapel Hill for college, and after graduating with degrees in chemistry and mathematics, enrolled in the MD/PhD program at Stanford University, where he joined the laboratory of the physical chemist Harden McConnell.

For his doctoral studies, Rabinowitz elucidated how naturally occurring antagonists of the immune system's T cells exert their effects. The experience, he says, was similar to what he does now—a mix of wet lab work and writing equations—and it

convinced him he wanted to be a researcher rather than a doctor. “Being a great doctor means making mistakes as rarely as possible, and that’s not my natural mindset,” he says. “I want to push the frontiers, to challenge dogma and do things differently. Lab science is really good for me that way. I love that feeling of coming in every day, dreaming up a new experiment or calculation and hoping that it will pan out and yield a nugget of scientific gold. Such nuggets are rare. But the search is thrilling.”

After receiving his PhD in 1999, and while completing his medical studies, Rabinowitz began looking around for a biotech startup to join. Aware of his search, McConnell called him into his office one day. “He had this very scratchy, deep voice,” Rabinowitz recalls. “He said, ‘Josh, if you want to do startups, look at this thing.’ And he pulled this little metal canister out of his pocket and said, ‘My friend, a great entrepreneur, has a dream of using this to deliver pharmaceuticals. If you’re interested, call this number.’”

That number belonged to the legendary Silicon Valley biotechnologist Alejandro Zaffaroni. A leading contributor to the invention of the birth control pill, the nicotine patch and other slow-release drug delivery systems, Zaffaroni now saw great potential in rapid drug delivery and wanted to develop an inhaled delivery device. Rabinowitz began in 2000 working out of Zaffaroni’s family office and, six months later, convinced his employer to launch a biotech company. It was named Alexza, and it started out in the basement of another biotech, Surromed.

Leading the new company’s product discovery efforts, Rabinowitz oversaw the growth of a more than 50-person R&D team, eventually leaving in 2004 as the company grew into a clinical development phase. Within a year of his departure, Alexza had four inhaled drug delivery systems in trials, one of which is now in the market for the treatment of acute agitation in psychiatric disorders.

“Being a great doctor means making mistakes as rarely as possible, and that’s not my natural mindset. I want to push the frontiers, to challenge dogma and do things differently.”

## COOL RESULTS

Ready to reenter research, Rabinowitz headed back east, where his future wife Emily Pronin—a research psychologist he first met at an open house, when the pair were graduate students apartment hunting in Stanford—had been recruited as an assistant professor in the psychology department at Princeton University.

As it turned out, David Botstein, the new director of the Lewis-Sigler Institute for Integrative Genomics at Princeton, was looking for a chemist who could teach chemistry from an interdisciplinary perspective and, on the research side, develop systems for the quantitative analysis of metabolites. Rabinowitz, whose PhD research had involved some of that invention and analysis, fit the bill. He was hired in 2004 as an assistant professor at the Institute, where he started a metabolomics group. “I started at ground zero,” says Rabinowitz. “I’d

“We were doing a lot of collaborations because, all of a sudden, people were like, ‘Oh, there’s this new thing we can measure that seems like it can give cool results.’”

never even touched a mass spectrometer before.”

Used to identify and quantify molecules in a sample, the mass spectrometer is the workhorse of metabolomics. Rabinowitz began with a relatively simple machine and, rather than look for as many metabolites as possible, focused on about 100 that were well known and of fundamental importance to biology. “It was a very different approach from what others were taking,” he says. He also picked a simple problem for starters, beginning with an analysis of nitrogen metabolism in the bacterium *E. coli*, before moving on to increasingly sophisticated analyses of yeast metabolism in collaboration with Botstein and others.

“We were doing a lot of collaborations because, all of a sudden, people were like, ‘Oh, there’s this new thing we can measure that seems like it can give cool results,’” says Rabinowitz. One of those people was the virologist Thomas Shenk, with whom Rabinowitz launched the field of viral metabolism when the pair reported in 2006 that, to replicate, the cytomegalovirus—a

common human pathogen—alters glucose metabolism in its host cells to drive the synthesis of fats.

## INTO CANCER RESEARCH

Another, of course, was Craig Thompson. Cancer cells are often forced to rewire their metabolism to generate the raw energy and molecular building blocks required for their ceaseless proliferation. Rabinowitz’s technologies offered a more comprehensive picture of the adaptations that make this possible, and he was soon collaborating with a growing list of leading cancer researchers, developing new metabolomic methods, technologies and analytical systems that extended well beyond the measurement of just the fundamental 100 metabolites with which he’d started.

Further, his analyses were now distinguished by their focus not only on the comprehensive identification and measurement of metabolites, but on their flux as well. “Flux is the most important output of metabolism,” says Rabinowitz. “If we see a metabolite that’s gone up or down, we need to know if it’s up because consumption decreased or production increased. If you want to target a pathway to treat cancer, you want to target the one that’s hyperactive to produce the required metabolite, not where the metabolite builds up because its consumption is gone.”

In 2008, Rabinowitz was included in a proposed Stand Up 2 Cancer (SU2C) Dream Team seeking to explore the metabolic disruption of pancreatic cancer for therapy. That was how he met Ludwig Scientific Director Chi Van Dang, a slated teammate then at Johns Hopkins University who had offered to critique his SU2C grant proposal. “I remember Chi pointing to one part of my proposal and saying, ‘you have to explain why someone should care about this, not just what measurement you’re going to make,’” says Rabinowitz. “It was really good advice,



Photo by Flynn Larsen

which impacted how I wrote all my grants going forward. And it was critical advice because getting that SU2C grant transformed my career due to all that I learned from my grant teammates.”

Closer to home, Rabinowitz also began working with Princeton colleague Yibin Kang, and Eileen White of the Rutgers Cancer Institute of New Jersey, both now founding Members of the Ludwig Princeton Branch (see *accompanying profiles, pages 22 and 30*). He and Kang first collaborated on a small but pioneering study, on metabolic changes in metastatic cancer cells, which was published in 2010. Around the same time, White got in touch with him to discuss how his expertise might aid her in exploring autophagy, a process of self-cannibalization that White had discovered many cancer cells depend on for survival and growth.

In 2011, Rabinowitz and White described how

cancers driven by the oncogene Ras rely on autophagy for core metabolic processes essential to energy generation. Over the next several years, he and White detailed the role of autophagy in the maintenance and progression of Ras-driven lung tumors, confirming its candidacy as a metabolic dependency that might be disrupted for cancer therapy.

“Autophagy is important for nutrient supply, for eliminating antigens and, at a whole-body level, in some complicated way, setting immune tone,” says Rabinowitz. “From the metabolism perspective, it’s one noncanonical way of getting nutrients.”

Another, Rabinowitz would show in collaborations with colleagues on the SU2C dream team, is a process known as macropinocytosis. “This is the cancer reaching out arms and doing autophagy to stuff outside the cell,” says Rabinowitz. “It

grabs whatever surrounds it, takes it in and chops it up.” Rabinowitz and his colleagues on the SU2C team subsequently demonstrated its importance in Ras-driven tumors and pancreatic cancer, suggesting another metabolic dependency to explore for cancer therapy.

## **METABOLIC FUNDAMENTALS**

Meanwhile, Rabinowitz and his team were also developing new experimental and computational methods to track the flux of molecules of fundamental importance to metabolic processes. One of them was NADPH, which is second in importance only to the molecule ATP as a cellular currency of energy. In 2014, they reported that a previously unknown source of this exhaustively studied energy molecule is folate, a vital nutrient and co-factor in many metabolic reactions—and, as it happens, a target of the oldest of chemotherapies.

Over the next year, Sean Morrison of the University of Texas Southwestern Medical Center built on these findings to show that this mode of NADPH generation is of functional importance to metastatic cancer cells. Rabinowitz and his team have since further elucidated links between folate metabolism and NADPH. More recently, they’ve been generating and evaluating targeted small molecule inhibitors of mitochondrial folate metabolism—which is hyperactive in multiple cancers—as potential cancer therapies.

By the middle of the last decade, Rabinowitz was also moving beyond cell cultures and examining systemic metabolic flux in living animals. In collaboration with White’s lab and others, his team examined the fate of a circulating metabolite known as lactate in both healthy mice and those with pancreatic and lung cancers.

Lactate is produced by the breakdown of the sugar glucose, a molecular building block of carbohydrates, through a pathway known

as glycolysis. This pathway either produces pyruvate—which can be shuttled through a series of reactions known as the TCA cycle to produce energy—or lactate, which is secreted into the circulation. Produced by cells starved of oxygen, like over-exerted muscle or cancer cells at the heart of a tumor, lactate was long believed to be a waste product primarily cleaned up by the liver.

Rabinowitz and his team reported in 2017 that lactate is in fact a major source of fuel for cells throughout the body. “Different parts of the body work in concert to metabolize carbohydrates,” Rabinowitz explains. “This occurs in two main steps: conversion of glucose to circulating lactate and burning of lactate. The second step, of lactate burning, is a generic process that happens everywhere in the body, while the first step is a special process that happens preferentially in the certain types of muscle fibers, the brain, activated immune cells, and cancer. The universal role of lactate as a fuel means that the whole body, not just liver, will clean up any ‘extra’ lactate made by tumors. At the same time, lactate in the tumor microenvironment is a fuel available to both cancer cells and immune cells.”

Rabinowitz has also continued collaborating with Kang, an adept developer of mouse models for the study of metastasis. The pair reported in 2016, for example, that an amino acid known as serine is the source of single carbon units that are used to generate the bases of DNA in proliferating cells, and that two different enzymes can perform the same key reaction in that process using folate. In a more recent study co-authored with Kang, Rabinowitz’s team showed that an enzyme ordinarily essential to maintaining NADPH levels in cells can be circumvented in breast and lung cancers driven by the K-Ras oncogene. In other tumor types, such as lung cancers driven with KEAP1 mutation, this same enzyme is essential and a promising drug target.



Photo by Flynn Larsen

Work on pancreatic cancer, meanwhile, has pulled Rabinowitz closer to the clinic. He is already involved in a clinical trial examining the effect of a ketogenic diet—high fat, moderate protein and very low carbohydrate—on triple chemotherapy for pancreatic cancer. “If we succeed in completing this trial, it’ll be the first adequately powered, randomized trial of dietary intervention to augment cancer therapy,” says Rabinowitz. At the Rutgers Cancer Institute, where Rabinowitz directs a metabolomics center, his team is also applying its technologies to study the metabolism of glucose in pancreatic cancer patients.

Ludwig Princeton’s partnership with the Institute will play a big part in its work, especially as discoveries made at the Branch are translated into clinical applications. One challenge Rabinowitz has for the Branch, for example, is to explore cachexia,

the deadly wasting that accompanies advanced cancer, with an eye to developing preventive treatments. “Ludwig support is enabling my lab both to push frontiers of metabolism measurement technology, and to engage more intensively with clinicians to understand the metabolic vulnerabilities of human cancer and the metabolic needs of cancer patients,” says Rabinowitz.

What really captures Rabinowitz’s imagination is the establishment of a scientific foundation for dietary interventions to prevent and treat cancer. “It’s amazing how many people—since hearing about the Ludwig Princeton Branch—have told me about their struggles with cancer, and they just didn’t know what to eat and wanted to make wise choices, but didn’t have the guidance,” says Rabinowitz. “I want to fix that.”

Few are better suited to the challenge. ■