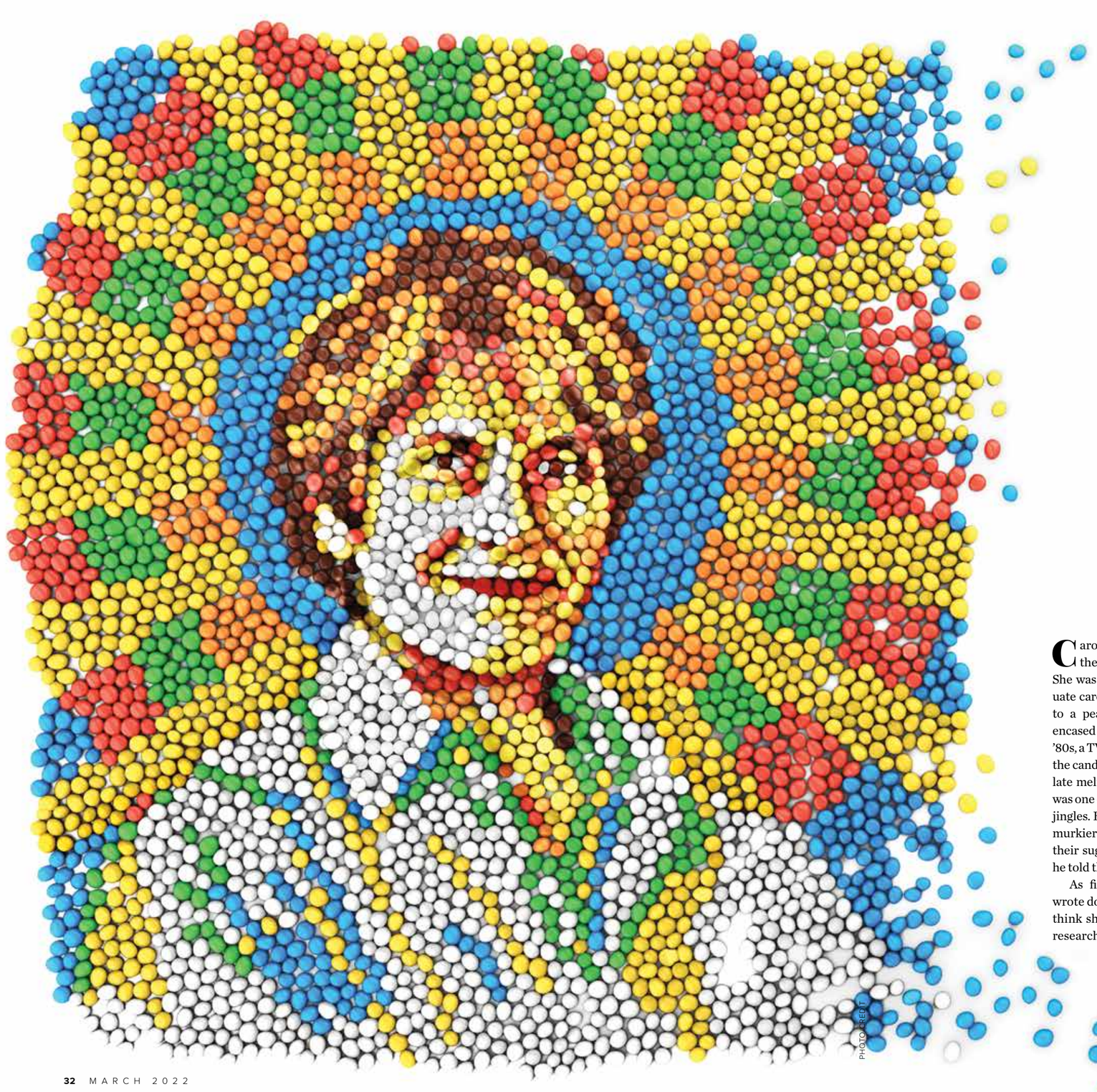

PHOTO CREDIT

Life Is Sweet

Carolyn Bertozzi wanted to see and work with the sugars that coat our cells. So she created a whole new approach to chemistry.

That was just the beginning.

By Sam Scott

Carolyn Bertozzi's introduction to the sugars that coat the outsides of our cells was short and, well, sweet. She was taking a biology course early in her undergraduate career at Harvard when the professor likened a cell to a peanut M&M. Both, he told the students, were encased in a sugar coating. At this moment in the mid-'80s, a TV-obsessed America couldn't help but know about the candy's sugary exterior. "The milk chocolate melts in your mouth, not in your hand" was one of the era's most familiar commercial jingles. But why our cells were similarly dressed up was a murkier matter even to Bertozzi's professor. Perhaps their sugary exteriors also provided a protective barrier, he told the class. No one knew the extent of it.

As first impressions go, it wasn't much. Bertozzi wrote down the analogy and moved on with no reason to think she had just gotten the first taste of the realm of research that would make her a scientific superstar: a MacArthur "genius" at 32, the first woman to win the $500,000 Lemelson-MIT Prize, and a perennial presence in future-Nobel-laureate guessing games. It was later, in graduate school at Cal, that Bertozzi would become steeped in glycoscience.

Over the ensuing decades, the M&M metaphor has stayed with Bertozzi—a chemical biologist who directs Stanford's Chemistry, Engineering and Medicine for Human Health institute, aka ChEM-H—as a way to illustrate a still underappreciated fact: Our cells—like every cell on Earth—are shrouded with sugars as surely as the famous colorful confections, and yet we know little about our own candy coating. Bertozzi has spent her career trying to understand, explore and exploit the mysterious, apparently indispensable nature of these sugars, with implications for how we treat everything from common cancers to rare genetic syndromes.

Illustration by hitandrun

‘If we’re successful, history will look back on this field and wonder how people could have been so blind. But that’s how it is.’

Her enthusiasm hasn’t exactly been contagious. The field may have a disarmingly familiar name—*glyco-* is from the Greek word for sweet—but it has been neglected in part because of its complexity. The sugars on our cells form branching chains called glycans that are daunting in structure. Whereas life’s other building blocks, like proteins, unfold according to genetic templates, the glycans are ultimately products of metabolism that can develop in any number of ways. A small number of them can combine in paralyzingly plentiful ways, and even then, their final form is defined by a variety of enzymes. Most researchers have preferred to focus on fast-advancing areas like RNA, DNA and proteins, leaving sugars as the dark matter of the biological universe, says Ajit Varki, a glycoscientist and physician at UC San Diego. Everyone knows they’re exerting untold influence, but hardly anyone studies them.

Bertozzi is trying to drag sugars into the light. After nearly two decades as a professor at UC Berkeley, she uprooted her lab in 2015 in search of resources at Stanford—including an on-campus hospital and the school’s entrepreneurial culture—that would better enable her to bring her academic work into the real world. In the past six years, she has co-founded eight start-ups, most working on sugar-based diagnostics or therapeutics, including one, Grace Science, directly inspired by the dire need of a young daughter of Stanford alums. Perhaps none is more intriguing than Palleon Pharmaceuticals, which makes a sugar-targeting drug that Bertozzi hopes will usher in a new form of cancer treatment—and raise the flag for glycoscience. The drug begins clinical trials this year.

“If we’re successful, history will look back on this field and wonder how people could have been so blind,” Bertozzi says. “But that’s how it is. Glycoscience has been a blind spot in medicine and in biology and in biopharma.”

Sugarcoated

As is probably obvious, the sugars on your cells don’t bear much resemblance to C&H crystals, or whatever goes in your morning coffee (or, frankly, to the exterior of M&M’s). Glycans are long, swaying, branching chains of complex carbohydrates that, in humans, are formed from nine types of simple sugars, or monosaccharides. (Think that’s complicated? In bacteria, glycans are composed of hundreds of types.) Together, these chains sprout from the surface of a cell like an overgrown garden, creating the blurry halo that often surrounds textbook photos of a cell.

But while glycan gardens might seem wild, on healthy cells they create distinct patterns—one liver cell will have a similar sugary decoration to another—that proteins, bacteria, viruses, cells and other passersby decode to know who they are dealing with. Sugars are how sperm know they’ve reached the egg, and why your immune system would rise in rage at a transfusion from the wrong blood type. Bertozzi likens the sugars to a cell’s barcode, whereas Laura Kiessling, a glycoscientist at MIT, compares glycans to a cell’s identification card (it helps to stay agile with metaphors when considering glycans). As with the ID in your wallet, there are bad actors who’d like nothing better than to forge it.

For decades, scientists have known that the glycans on a variety of cancerous tumors undergo dramatic changes distinguished by the proliferation of a particular sugar called sialic acid. But it was not clear to what end. During the past decade, Bertozzi’s team, as well as others, have revealed sialic acid’s insidious trick. As the sugar thickens on a tumor, it lulls the immune cells—which otherwise might find and fight it—to sleep. In another context, sialic acid’s calming influence—essentially a barcode signal indicating “self”—might be a virtue, quelling autoimmune attacks, for example. But cancer is using the sugar for nothing so noble. It’s cloaking itself in a false identity to hide from our immune protectors. In response, Palleon has created a product that scythes the sialic acid with a “molecular lawnmower,” stripping away the cancer’s mask and exposing it to the newly awakened immune system. Or that’s the goal.

In concept, the drug is similar to immunotherapies developed in the past decade, which likewise try to stop cancer from activating “off” switches within our immune system. (With its awesome, potentially self-destructive firepower, our immune system absolutely requires fail-safe buttons.) Perhaps the best-known immunotherapy patient is former U.S. president Jimmy Carter, who was all but pulled from the grave by a treatment called Keytruda after metastatic melanoma spread to his brain and liver.

But only a minority of eligible cancer patients respond to such treatments. Bertozzi is hopeful that Palleon can significantly expand the number, not least because the company believes more tumors cloak themselves in sialic acids than exploit the protein receptors blocked by Keytruda. Varki, who has no connection to Palleon, says the approach “has a lot of promise,” though Bertozzi acknowledges success is far from assured. Any drug that reaches human tests has excelled in lab and animal models, yet the vast majority fail to clear the remaining hurdles. Nevertheless, she is optimistic. “We at Palleon, and more broadly the glycoscience people, we’re quite bullish about this,” she says.

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A light-up zebrafish

Her foray into entrepreneurship adds a new chapter to a career already distinguished by a talent for building new chemical tools and methods. Indeed, when people lay their bets on Bertozzi’s winning a Nobel Prize, it’s often for her most famous tool—bioorthogonal chemistry, a term she coined for a field her lab pioneered that allows scientists to pursue chemical reactions inside a living system without disturbing it.

Imagine a white-coated chemist mixing chemicals in a sterile flask in a sterile lab where heat, light, acidity, temperature, etc., are all under her total control. Now imagine her trying to pursue similar reactions in a living cell where nature dictates the environment. Classically, it would have been impossible, especially without harming the cell. But over the course of more than a decade, Bertozzi’s lab found better and better ways to do just that—by developing very fast, very selective and very nontoxic reagents that react with each other—and only each other—amid the distractions of a living system.

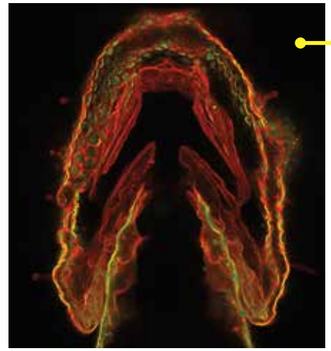
Bertozzi developed the approach, originally, out of frustrations with the difficulties of studying glycans. In the late ’90s, no imaging technology was available to see the sugars in vivo, as was possible with proteins or nucleic acids, an obstacle that was preventing Bertozzi from pursuing ideas like using changes in the glycans as diagnostics for diseases.

And so Bertozzi devised an elegant solution born of an uncommon expertise in both chemistry and biology. She duped cells into digesting an unnatural sugar so similar to the real thing, the cells unquestioningly added it into their glycan coats. This unnatural sugar was utterly inert to its new surroundings, but it was extremely reactive with another chemical it was then exposed to. And this second chemical came carrying fluorescent probes that would glow to reveal their new homes. It was like a benign Trojan Horse. The cells welcomed in gifts of sugar and gave them pride of place in their glycan coats, and the sugars turned around and provided a landing pad for tools that allowed Bertozzi and colleagues to see and manipulate the cell’s glycans. The cells hadn’t been harmed or significantly affected, but their sugary canopies had been exposed.

“Think of it as finding needles in a haystack,” says Thomas Cech, a biochemistry professor and Nobelist at the University of Colorado who helped select Bertozzi as an investigator for the Howard Hughes Medical Institute in 2000. “With Carolyn’s chemistry, every needle now glows in the dark.”

Bertozzi and her students and postdocs painstakingly honed the process for years, leading to a moment in 2008 when they released a paper showing the glycans of an embryonic zebrafish lit up like a Christmas tree, the first time the glycome of a living organism had been imaged. It was, in the words of chemist M.G. Finn, like a “global positioning system” for tracking sugars through organisms.

The technique continues to open new windows into the glycan world. Last year, Ryan Flynn, PhD ’15, MD ’17, a postdoc in Bertozzi’s lab, used biorthogonal probes to reveal glycans dangling from ribbons of RNA on cell surfaces, upending accepted



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wisdom that glycans were rooted in either proteins or fats embedded in the cell. RNA, meanwhile, was supposed to exist only within a cell.

“It’s really a bombshell because the discovery suggests that there are biomolecular pathways in the cell that are completely unknown to us,” Bertozzi, the study’s senior author, said at the time. She also noted the magic of interdisciplinary collaboration: “Ryan is RNA, I’m glycans. We have completely different backgrounds.” The discovery may ultimately have implications for the treatment of autoimmune diseases.

Indeed, from the start, it was clear that bioorthogonal chemistry had broad applications. Bioorthogonal approaches have been used to observe and manipulate lipids, proteins and nucleic acids, and to stitch together antibody therapies with drug payloads, as well as to engineer and manipulate glycans with a precision previously associated with the molecular surgery of recombinant DNA. Most significantly, it has changed where chemistry is possible. “What Carolyn’s vision has done, she’s replaced the glass flask with a cell, with a zebrafish,” says Neal Devaraj, PhD ’07, a professor of chemistry and biochemistry at UC San Diego, who was earning his doctorate in electrical chemistry at Stanford when he saw Bertozzi speak on her zebrafish work, a “mind-blowing experience” that steered his career toward studying how nonliving matter such as simple organic molecules can assemble to form life. “That is a fundamental paradigm shift in the way we think about doing chemistry.”

And one day, the chemistry Bertozzi enabled may occur inside you. In October 2020, Shasqi, a start-up that Bertozzi advises, began the first use of bioorthogonal chemistry in humans during clinical trials of a targeted cancer treatment. In Shasqi’s proposed therapy, a tumor is injected with a biopolymer before the patient receives an “encaged” dose of chemotherapy. Only upon reaching and reacting with the polymer at the tumor is the chemotherapy released. The goal, Bertozzi says, is to deliver the chemo with a level of precision that enables higher doses while protecting the rest of the body from chemotherapy’s toxicity.

There are plenty of other reasons pharmaceutical companies might want medicines to assemble in the body, Devaraj says—size being one. Neurological therapies, for example, may better slip past the filter of the blood-brain barrier if they are transmitted in components, he says, uniting only once they’ve reached the brain. He expects many companies to follow Shasqi’s lead. A Nobel Prize for work of such influence, he says, is very plausible. “I may even argue it’s just a matter of time.”

O, chem

Before she was a rock star scientist, Bertozzi was almost a rock star, or at least she came closer than your average member of the National Academy of Sciences. A play-by-ear pianist who still relaxes by figuring out pop tunes, Bertozzi was the keyboardist in a hair metal band at Harvard called Bored of Education, alongside guitar wizard Tom Morello, who later founded Rage Against the Machine and Audioslave. The world is a poorer place

Bertozzi had been flirting with becoming a doctor when she fell so hard for O-chem her sophomore year that she couldn't tear herself away from her textbook long enough to go out on Saturday nights.

for the dearth of photographic evidence of their collaboration. (Bertozzi has photos of Morello in leather-panted glory but not of herself. She consoles the reader by saying her hair was much as it is now.) And she sometimes jokes about missing her chance to follow Morello to Los Angeles.

In reality, she was probably never destined for an artistic career. She was the middle daughter of an MIT physics professor and of a secretary turned stay-at-home mom who had been denied the chance to go to college by her parents and who was determined that her own daughters would receive a far different message. Bertozzi grew up in a household where academics were central, grad school was assumed, and science and math reigned supreme. (Her dad, an inveterate builder and tinkerer, also fostered a valuable DIY ethic by having Bertozzi do masonry and lay concrete.) In Bertozzi's telling, it was her elder sister, now a mathematics professor at UCLA, who was the obvious brain. Her own application to Harvard, she says, was fortified by her soccer talents, although she didn't play there for long. Once practice and games conflicted with labs and class, there was only one outcome.

Plus, there was soon organic chemistry to think about. The course is famous for weeding out premeds, and so it did here, albeit not for the typical reason. Without any clear career ambition, Bertozzi had been flirting with becoming a doctor when she fell so hard for O-chem her sophomore year that she couldn't tear herself away from her textbook long enough to go out on Saturday nights. A torture to many was pure pleasure for her. It just made intrinsic sense.

Alas organic chemistry—or its scholars of that era—didn't love her back with equal gusto. Despite being the top student in the course, she struggled to get a research job on campus, a fact that to her seemed suspiciously related to her being a woman in an overwhelmingly male department. She ended up working for a junior faculty member in physical chemistry—ostensibly a less prestigious post—but he challenged and championed her, and her senior thesis project, in which she built from scratch something called a photoacoustic calorimeter, won a prize. (It also reinforced a fondness for the road less traveled—at Cal, she chose another junior faculty member as an adviser, the late Mark Bednarski, who introduced her more deeply to sugars. As a postdoc, she worked in a biology lab, a disciplinary zigzag then frowned upon.)

It wasn't just gender that marked Bertozzi as an outsider at Harvard. It was also sexuality. In 1986, during her sophomore year, the Supreme Court handed down the *Bowers v. Hardwick* decision upholding an antisodomy law and creating an unsettled feeling in Bertozzi that she, as a lesbian, had been criminalized. Maybe there wasn't reason for concern in Cambridge, Mass., but what about 35 miles away in New Hampshire or whatever jurisdiction she might cross into? Her decision to go to grad school at Berkeley wasn't only about the school's excellence in chemistry. The organic chemistry department had a small but significant female presence. And it was a stone's throw from the most gay-friendly city in the country.

A generation later, she still feels a duty and a desire to be a role model as a lesbian scientist and to talk about struggles she lived through, from the AIDS crisis that shaped her early years in the Bay Area and motivated her goal to create medicines to the fight for marriage equality that provided a discordant soundtrack to some of her most fulfilling years. Bertozzi has three sons with her wife, the first born just before California voters approved Proposition 8, which banned same-sex marriage in the state. She remembers feeding him when he was 2 months old, with anti-gay marriage ads blaring on the TV and the anxiety of not knowing whether her parental rights would survive the election. "I think it's important for me to bear witness," she says. "I never want to be like, 'Well, all that is behind me; now I'm just like everybody else legally.' No, I don't think that does anyone a service."

To save Grace

Service is a big part of what drives Bertozzi. She is renowned for her commitment to her students. Her first doctoral advisee, Lara Mahal, says Bertozzi demanded a lot—Mahal almost quit in protest at one point—but gave back just as much. "It's part of why she is incredibly successful: She is so giving and generous, and those are not words used with many highly successful scientists," says Mahal, now the Canada Excellence Research Chair in Glycomics at the University of Alberta. "It has always been a two-way street with her."

In Silicon Valley, it's easy to perceive her surge into start-ups as a play for financial glory, an assumption that makes Bertozzi laugh. The road to riches in biotech is long and unlikely, she says. "If you really want to make money, go be a hedge fund manager or something," she says. "Don't be an academic founder of a biotech." Instead, she says, her emergence as serial entrepreneur is about making sure her lab's work has the maximum impact possible. Sometimes, there's no better way of doing that than doing it yourself. And sometimes it's just what fate seems to want.

Bertozzi wasn't even finished unpacking her moving boxes in September 2015 when she got an out-of-the-blue email from a stranger, Matt Wilsey, '00, MBA '08, with an opportunity for research funding. It was exactly what she had come to Stanford for, yet the details were like nothing anyone could have anticipated.

Wilsey's daughter Grace had been born in 2009, and a host of troubling symptoms had unfolded—weak muscles, difficulty eating, tearless eyes, poor sleep, development delays and more—that would confound scores of specialists over the coming years. It was only when she was 3 that she was diagnosed with a deficiency on a single gene, known as NGLY1, which was causing a vanishingly rare disorder that was completely unstudied and would almost certainly remain so. The family had been able to connect with a handful of others around the world enduring the same odyssey, but the number of known patients was in the double digits. In the harsh logic of business, there was almost zero incentive for anyone to look for a cure.

In 2014, Wilsey and his wife, Kristen, '03, took the onus on themselves to change that, creating a foundation to fund research with support from friends, loved ones and other contributors. Wilsey had already recruited dozens of scientists when multiple people recommended he try Bertozzi. The NGLY1 gene was known to be affiliated with sugars, but nobody knew why a deficiency there would be so devastating.

For Bertozzi, the decision to get involved wasn't difficult. As a mother of children around Grace's age, she could feel the terror of such a mysterious, possibly fatal diagnosis. As a glycoscientist, she knew she had rare skills to help. And as someone intent on translating her research into the real world, she had found exactly what she came for. She convened a group of postdocs and students to collaborate.

Results were not long coming. Bertozzi's lab drew a bead on NGYL1's role in initiating a cellular-garbage recycling process that's responsible for chopping up old and damaged proteins. In the cells of Grace and the others, NGLY1 wasn't there to start the process—a step that involves removing a sugar from the molecule that does the degrading. And it was leading to a destructive pile-up of trash.

The particulars of the findings were transformative, Wilsey says. Not only did they have direct implications for Grace's disease, but the mechanism had relevance for degenerative diseases, like Parkinson's, as well as for cancer, where conversely you might want to thwart garbage removal to cause malignant cells to die. These were diseases that would attract investment dollars that could transform the scale of their efforts. "All of a sudden it opened up this whole new universe," Wilsey says. "I mean, it was like we were stepping through the looking glass."

In 2017, Wilsey and Bertozzi co-founded Grace Science, intent on putting the research into action. The company expects to begin clinical trials on a gene therapy for NGLY1 in early 2023, an agonizing wait for the Wilseys but no time at all compared with the norm. "It's lightning-fast in the drug-development world," Wilsey says.

There are currently about 100 known people with NGLY1 deficiency in the world, and Bertozzi has met many of them through gatherings organized by the Grace Foundation. Although her career brims with accolades, connecting personally with people whose health may someday benefit from her work is "one of the greatest privileges of my career as a scientist," she says. Success on their behalf would be sweet. ■

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