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PROFILE

James Inglese: Uniting Biology and Chemistry in High Throughput

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Ever since the Human Genome Project was completed in 2003, scientists have been at odds over how best to unlock the potential for translational research encoded in the newly sequenced 20,000-odd genes. For James Inglese, Ph.D., the answer is simple: to solve this tough biological problem, look to chemistry. As deputy director of the National Institutes of Health Chemical Genomics Center (NCGC), Inglese and his colleagues use high-throughput screening (HTS) to help other investigators identify small molecules that might make novel and effective tools for studying proteins, cellular functions, and biological processes involved in physiology or disease. After winding his way from academic laboratories to small biotech and big pharma, Inglese uses his unique perspective to guide a team of about 50 scientists, helping them to develop powerful and efficient assays and to make the most of HTS hits through chemistry optimization. These efforts, provided as a government-funded resource for researchers selected several times a year from around the world, expand the options for small academic laboratories and biotech firms that cannot afford a large HTS operation on their own. By taking HTS in this new direction, Inglese and his colleagues are tapping the genome's potential, one 1536-well plate at a time.

Chemistry Set to Chemistry Laboratory. Inglese was born in 1962 in Morris Plains, NJ, to a father who owned a barbershop and moonlighted as a nightclub accordion player and a mother who was a full-time homemaker. Though his parents were not highly educated, says Inglese, they encouraged him to do whatever he found interesting, whether or not they understood his passion. Consequently, when he started showing an interest in science at age 6 or 7, his parents bought him a Gilbert chemistry set as a gift. "It was a relatively young age to get that kind of toy, but my parents didn't know that," says Inglese.

Before long, his small chemistry set had blossomed into a large chemistry laboratory in his family home's basement, complete with a gas utility line for supplying his Bunsen burner and a fume hood for pulling out noxious gases. There, Inglese performed hundreds of experiments gradually growing in complexity, from preparing nitrocellulose from cotton soaked in nitric and sulfuric acids to synthesizing lophine and watching it chemiluminesce with a greenish glow. He even performed some elementary nuclear chemistry experiments, watching the formation of water vapor contrails from subatomic particles as they zipped through a homemade cloud chamber. Inglese remembers that each of these experiments required lots of patience, sometimes taking hours, days, or weeks to work. "These experiments weren't easy, but when they were finally successful, it was worth it," he says. "I came to appreciate the end product and understand that real payoffs don't come from instant gratification."

As Inglese progressed through high school, physics and chemistry teachers encouraged his interest. By the time he was ready to apply to college, he was certain that he wanted to pursue chemistry as a career. Rather than learn science as part of a broader liberal arts program, Inglese knew that he'd rather focus specifically on chemistry. To that end, he applied and was ac-

cepted to Rensselaer Polytechnic Institute in Troy, NY, a school focused on science and engineering.

Though he hadn't been interested in biology in high school, Inglese was required to take several biology classes at Rensselaer. Eventually, he found himself in a mechanistic biochemistry class taught by medicinal chemist James K. Coward. Inglese notes that this class is typically taught by rote memorization. Instead, Coward "transformed biology into a logical process driven by chemistry," remembers Inglese. "I finally saw that one of chemistry's most important uses is in nature, to generate biology."

With his newfound appreciation for the life sciences, Inglese began spending summers in Coward's laboratory. His research project focused on methotrexate (MTX), an anticancer compound that works by inhibiting the metabolism of folate. Some of the drug's toxicity to normal cells is thought to be due to γ-glutamylation of MTX, which helps to retain and compartmentalize the drug in cells. Inglese worked with Coward and his colleagues to create an analog, γ-fluoromethotrexate, which replaced one of the molecule's hydrogen atoms with fluorine, effectively preventing γ-glutamylation. Though the molecule did not progress through the drug pipeline, it did earn Inglese his first publication (*1*) and a new understanding of the importance of publishing and disseminating work in science. "I didn't have any prior knowledge of the pathway for developing a career in science. I just kept doing things because I liked them," says Inglese.

After that first paper was on its way to being published, he recalls, he was "hooked" into taking his education to the next level. On Coward's suggestion, Inglese targeted his search for graduate schools to Pennsylvania State University in University Park and the laboratory of Stephen J. Benkovic, a chemist who specializes in enzyme catalysis. Benkovic's work has often focused on dihydrofolate reductase, the enzyme inhibited by methotrexate, making Inglese's experience in Coward's laboratory a good fit for a new recruit.

Collecting Collaborators. Inglese joined Benkovic's laboratory in 1984 and soon started working on purifying and characterizing the mechanism of glycinamide ribonucleotide transformylase (GAR TFase) using physical and kinetic methods. Early in the project, Inglese isolated the enzyme from fresh chicken livers, going right to the source to gather his materials. He remembers driving to a chicken coop in rural Pennsylvania on early mornings and meeting with a caretaker who fed the birds the high-protein diet necessary to optimize expression of GAR TFase in their livers. The caretaker schooled Inglese, who had no prior experience working with animal models, on how to sacrifice the chickens and remove their livers. "I really thought it was kind of creepy," Inglese quips.

Inglese was not only uncomfortable with dissecting chickens, he says he was also uncomfortable with how quickly his research was progressing. The livers did not provide much of the needed enzyme, and what little he did isolate quickly lost activity, impeding his experiments. After several tense months, he eventually made a connection with John M. Smith of Seattle Biomedical Research Institute, who had cloned and over-expressed the *Escherichia coli* GAR TFase, which provided a ready and plentiful source of the enzyme. Over the next five years, Inglese designed and synthesized affinity labels and a multisubstrate adduct inhibitor for GAR TFase, elucidating a slow, tight binding mechanism for this inhibitor (*2, 3*). His work developing the inhibitor earned him his first patent, sold to the pharmaceutical company Burroughs Wellcome (now part of GlaxoSmithKline). Through frequent trips to visit the company in Research Triangle Park, NC, Inglese's interest in compounds with pharmaceutical potential was piqued.

As Inglese neared the end of his doctorate, he and Benkovic discussed possibilities for where he might go for his postdoctoral fellowship. Benkovic suggested several researchers with interests that coincided with Inglese's. Having done his own research into postdoctoral possibilities, Inglese suggested Robert J. Lefkowitz at Duke University Medical Center, a leader in characterizing G-protein-coupled receptors (GPCRs). These transmembrane proteins are targets for the vast majority of pharmaceutical drugs.

Inglese applied for several different postdoctoral fellowships. However, he did not hear back from Lefkowitz until he was just about to accept another offer. When Inglese answered his apartment phone one afternoon, Lefkowitz was on the other line, encouraging him to come visit. Inglese did. "After just five minutes of being with Lefkowitz, I knew this was where I wanted to go," remembers Inglese. "This is an example of a personality being so compelling and engaging and enthusiastic that I wanted to talk with him as long as he wanted to talk with me. The power of his personality drew me there, and it was one of the best decisions I ever made."

In the fall of 1989, Inglese started working on several projects in the Lefkowitz laboratory. Over the next five years, he succeeded in cloning rhodopsin kinase (RK) and expressing it in mammalian and insect cell lines (*4*). He and his colleagues determined that this visual enzyme is isoprenylated and α-carboxylmethylated (*5*) and that its light-dependent translocation to bleached rhodopsin is dependent on isoprenylation (*6*). Inglese also made strides in characterizing β-adrenergic receptor kinases and other G-protein-coupled receptor kinases (GRKs).

Besides succeeding in these projects, Inglese says that one of his greatest accomplishments from his postdoctoral years was gaining experience forging collaborations. "Lefkowitz used to tell me that I was one of

the most productive collaborators in his lab. 'You have a good hit rate,' he'd say," remembers Inglese. "In today's environment, collaboration is key. Being an island is very difficult."

During his postdoctoral fellowship, Inglese formed strong collaborations with Krzysztof Palczewski, who furnished purified RK used in its cloning; Ching-Kang Chen, whom he worked with on Ca^{2+}-dependent retinal protein-RK interactions; Richard T. Premont, whom he partnered with on the cloning of other GRKs; and H. G. Khorana, whom he worked with on expression methods of RK. He also collaborated with numerous other investigators on the multiple effectors of the βγ-subunit of the heterotrimeric G protein, such as with Lilly Jan's laboratory to investigate K^+ channel activation (*7*).

During this time, he also forged an enduring relationship with Fraser Glickman, then a laboratory technician at Burroughs Wellcome whom he met one night at a bar near Duke's campus and who introduced Inglese to the topic of protein isoprenylation. To this day, Inglese continues to collaborate periodically with Glickman, who is now the incoming director of the Rockefeller University HTS Resource Center.

Small Biotech to Big Pharma. Like many academic scientists, Lefkowitz often encourages his protégés to follow him into an academic career. However, a chance meeting with Nolan Sigal, VP of Biology at Pharmacopeia, Inc., a small biotechnology firm based in Cranbury, NJ, presented an opportunity for Inglese to return to the chemistry-biology frontier. "It took me some time to convince Lefkowitz that industry was the right direction for me to go in," Inglese says. With Lefkowitz's blessing, Inglese started working as a Pharmacopeia senior scientist in January 1995.

Inglese was only the 40th employee hired, and he remembers it as an exciting time for the relatively new biotech company. Over the next five years, he helped to recruit many promising scientists, including Glickman, whom Inglese hired as his first postdoctoral fellow after Glickman finished his Ph.D. The company was an early pioneer in the world of HTS and combinatorial chemistry, quickly growing a large chemical library and developing strategies and assays for screening the biological activity of these molecules (*8*). While at Pharmacopeia, Inglese and his colleagues developed several high-throughput screens for whole-cell assays of CXC and CC chemokine receptors (*9*). He also helped discover a rare potent interleukin-8 antagonist, one of Pharmacopeia's first internal program successes (*10*).

Though he was excited about his success in developing useful screens, Inglese says that he eventually became frustrated by the lack of resources inherent in working for a small biotech company. "We were developing this sophisticated technology for screening, but the production versions of the instruments we helped design were too costly for a small biotech company to invest in," Inglese says. After discussing his concerns with Lefkowitz, who continued to mentor him in his career, Lefkowitz encouraged Inglese to contact Berta Strulovici, another former Lefkowitz postdoctoral fellow, who worked at pharmaceutical giant Merck & Co., Inc., based in North Wales, PA. A short time later, Inglese received a job offer. He started working as a senior research fellow at Merck in August of 1999.

There, Inglese's work concentrated on developing assays to test the action of all Merck compounds entering HTS. The experience was an unexpected learning opportunity, remembers Inglese. "I look at it almost like another postdoc," he says. Over the next several years, Inglese gained experience in managing 11 other scientists and interacting globally with colleagues in the company. He also got plenty of practice developing innovative screening protocols for a variety of small molecules aimed at health problems ranging from clotting disorders (*11*) to hepatitis C (*12*).

By late 2003, Inglese's role at Merck was growing, and he was thriving on the resources at his disposal and the productive collaborations he was forming. "I never wanted to leave," he says. However, an unanticipated offer soon came his way. Several months prior, Inglese had received a call from Christopher Austin, a senior advisor to Francis Collins, the director of the National Human Genome Research Institute (NHGRI). As an extension of the NHGRI's pivotal work on the Human Genome Project, Collins was interested in starting an HTS operation on the National Institutes of Health (NIH) campus. Austin was one of Inglese's former colleagues; he had recently been recruited from Merck to NIH, and he wanted Inglese to attend a planning meeting to brainstorm ideas for an HTS-focused center.

The center's goal would be to provide government-funded assay development, HTS, and chemistry optimization on a scale equivalent to a large pharmaceutical company. Though scientists in academia and small biotechnology companies might benefit from such technology and expertise, very few have access to such extensive resources and know-how in their own laboratories. Inglese and other researchers who attended the meeting thought this was a worthy goal, but the NHGRI initially decided that it could not support the expense of developing the center. However, within a few months, funding became available through the controversial NIH Roadmap for Medical Research, a plan meant to support cross-disciplinary, translational research.

When Inglese was offered a position as one of the founding leaders of the new NCGC, he took time to consider the offer. He was comfortable at Merck, and his wife was expecting the couple's first child. However, he remembered some advice that Lefkowitz had given him during his postdoctoral fellowship. "Lefkowitz had said that no matter where you are, you should know what your dream job will be so if it comes along, you can consider it," Inglese says. Af-

ter mulling over the decision while on vacation with his wife, Inglese realized the position was startlingly close to what he'd consider a dream job. Wasting no time, he called Austin and Collins to accept the position as soon as he returned home. Inglese started as an associate investigator of the Genome Technology Branch of the NHGRI in May of 2004 and became deputy director of the NCGC in February of 2005.

Opening HTS Opportunities. Joining the NCGC has afforded Inglese some of the same exciting pieces of the jobs he enjoyed at Pharmacopeia and Merck. For example, because the NCGC was starting from scratch like Pharmacopeia was, Inglese has had the opportunity to recruit many talented scientists, including some of his former colleagues. For example, some of his first recruits were Doug Auld from Pharmacopeia and Wei Zheng and his wife, Menghang Xia, from Merck. Inglese also made several recruits from other companies, including Anton Simeonov from Caliper Life Sciences and Ron Johnson from Human Genome Sciences. Each of these scientists is now a group leader, working within interdisciplinary project teams of biologists, chemists, informaticists, and engineers to bring projects through the NCGC's probe discovery and development process.

Also, as with his time at Merck, Inglese has had access to resources and freedom to design new assays and screening protocols. This freedom led to one of the new center's earliest successes. Inglese explains that as the NCGC was first ramping up, there was no laboratory in which to work and no equipment on which to run their assays. Having no existing machinery to dictate the types of screens they'd have to run, the scientists had the opportunity to brainstorm ideas for new protocols. "With this novel situation where there was no existing infrastructure for screening, we decided that we didn't have to do HTS the way it was moving forward for everyone else," he says.

Consequently, the researchers developed a novel plan for saving time and resources while making initial screens more accurate. Rather than screening just one concentration of a compound, then confirming the activity and developing IC_{50}'s later based on initial "hits", why not screen multiple concentrations from the start?

In 2006, Inglese and his colleagues outlined the details of a new method that they named quantitative high-throughput screening (qHTS) (*13*). Using a 1536-well plate format, the researchers reworked standard HTS to test seven different concentrations, titrating from highest to lowest, of more than 60,000 compounds against pyruvate kinase, a well-characterized enzyme involved in energy metabolism. When the team compared their quantitative HTS results with those generated by screening the same chemical compounds with traditional, single-concentration methods, they found the new approach produced a much lower prevalence of false positives and negatives. Consequently, Inglese notes, the new method could save investigators time and money spent following up on unpromising leads. Also, because the technique reveals compound potency and efficacy from the start, investigators gain rapid access to initial structure–activity relationships and selectivity profiles built over the course of subsequent qHTS runs.

With the NCGC up and running for more than three years now, Inglese and his colleagues have now assisted more than 40 researchers develop and run HTS assays to advance their research (*14–17*). Many of them are like the first investigator whose assay was accepted by the NIH's Molecular Libraries Initiative (MLI) for screening at the NCGC: David Williams, a biochemist specializing in parasite–host interactions at Illinois State University in Normal.

Williams studies schistosomiasis, a chronic parasitic disease caused by flatworms of the genus *Schistosoma*. With only one drug currently used to treat this disease, and the parasites quickly developing resistance, Williams and other researchers are currently searching for new drug candidates to treat schistosomiasis. This parasite has a unique way to protect itself from damage inflicted by reactive oxygen species generated by its hosts' respiration and immune systems that involves an enzyme, known as thioredoxin glutathione reductase (TGR). Studies have previously identified this enzyme as a possible target to fight *Schistosoma* organisms, yet no candidate drugs effective against this enzyme had been identified.

To find candidate compounds, Williams applied to the MLI to search for candidates in the initiative's growing chemical library. "His proposed assay is one that may have been rejected, since it used cuvettes, but the MLI thought the NCGC had the expertise to handle it," says Inglese. To help Williams' assay become HTS-ready, Inglese and his colleagues converted Williams' protocol so that it was compatible with a 1536-well plate format. They also set Williams' assay to run with their new qHTS technique.

When the NCGC ran his assay, they found that phosphinic amides and oxadiazole 2-oxides were effective inhibitors of TGR. When the researchers tested one of each type of compound on cultured *Schistosoma* worms, they both killed 100% of the worms in concentrations safe to mouse cells. The compounds were also effective against the parasites in mouse models of the disease (*18*). Inglese notes that finding these compounds, now on their way to drug development, would not have been possible if Williams was working on his own. "This is a small lab that doesn't have HTS capability," Inglese says. "To make this kind of progress, researchers need to employ the equivalent of a big pharma early-discovery program. This is what we do at the NCGC."

What Inglese and his colleagues do not do *via* the MLI, however, is follow promising compounds further down the drug pipeline toward development. They also try to

avoid focusing solely on targets that are already heavily pursued by pharmaceutical companies, such as popular GPCRs. However, any researcher is welcome to submit an application for probe discovery (*19*), and Inglese and his colleagues routinely provide early advice on HTS assay development (*20*). Inglese notes that applications to the NCGC, *via* the MLI, are reviewed three times a year by volunteers from academia and industry, with assays that fit NCGC's HTS protocols and those with biological or chemical novelty given first priority.

With more and more opportunities to perform interesting assays arriving each submission period, Inglese says that the job never gets old. "My favorite part of this job is what has always been my favorite part of every job: designing new experiments, looking at data, and interpreting results. I really enjoy just being a scientist", he says.

—Christen Brownlee, Science Writer

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