Transforming BIOLOGICAL SCIENCE *and* BIOMEDICAL PRACTICE

In 2016, more than 53,000 people in the United States died from overdoses of opioid drugs. Many of these were illegal narcotics such as heroin and fentanyl. But the underlying cause of this epidemic is generally agreed to be widespread legal use (and perhaps over-prescribing) of opioid drugs such as oxycontin to control pain. People using such drugs develop a tolerance, meaning that they eventually need more of the drug to achieve the same effect and often turn to cheaper but more powerful illegal drugs such as fentanyl, which is 50 times more potent than heroin.

The epidemic of opioid overuse—both prescription medicines and illegal drugs—has become a national public health crisis. New understanding of how opioids and other drugs attach to and interact with cells may make possible medicines that stop pain but are neither addictive nor suppress breathing. *(Karen Foley Photography / Shutterstock.com)*

As a result of the last several decades of research into how our body's cells communicate with each other and into the structure of proteins and other large biological molecules, however, scientists now understand much more about how the opioid drugs cause their effects. It turns out that these drugs act on two different levers within cells in the brain: one suppresses pain; the other causes tolerance and suppresses breathing, which is what causes overdose deaths. Suppose it were possible to uncouple these effects and develop a new class of opioids that only suppressed pain? In fact, just such an effort is well underway, with several potential drugs nearing clinical trials. And while that may not have much impact on the immediate crisis, it could help eliminate the underlying cause of opioid deaths for the future.

These and other similar biomedical breakthroughs—including hundreds of drug candidates already in the pharma pipeline—depend in part on the increasing adoption by biologists of tools initially developed for physical science research. Of particular value in deciphering the structure of a complex molecule are very intense beams of X-rays, billions of times stronger than those in dental offices. The tiny wavelength of these X-rays enables scientists to see much smaller particles than is possible with visible light, so that they can pinpoint the positions of atoms in a molecule and thus help to identify its physical structure. The machines that generate such X-rays are huge, as large as a football field, and include dozens of individual beamlines that can manipulate the X-rays as needed for a given experiment. They are too large and expensive even for pharma companies, let alone university researchers. Instead, most of this research is done at one of the five X-ray sources supported by DOE's Basic Energy Sciences (BES) office—three located in California, one near Chicago, and one on Long Island. These shared research facilities were built originally to facilitate chemistry and materials science research—which they do—but biological scientists (supported by the National Institutes of Health or the pharmaceutical industry) now constitute the largest user group, underscoring the importance of these facilities for improving healthcare now that biomedical science increasingly focuses at the molecular level.

The intense X-ray facility—comprising the huge magnetic storage ring and dozens of individual beamlines for research—at Argonne National Laboratory that has enabled academic scientists to determine complex molecular structures and helped pharmaceutical scientists optimize new drug candidates for clinical trials. *(Mark Lopez / Argonne National Laboratory)*

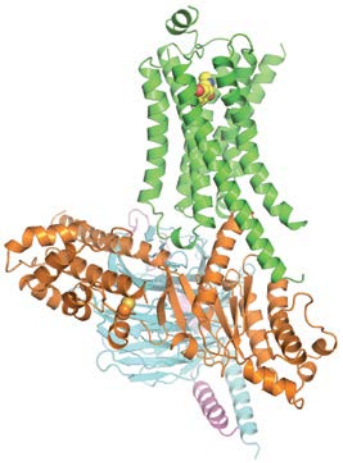
University researchers apply for time on a given beamline, typically to determine the structure of a molecule that is central to a particular biological process. One example is the cellular machinery that takes information from the cell's genes and uses it to manufacture the proteins that our bodies need to function. The research took over a decade, including repeated and increasingly high-resolution X-ray analysis at shared research facilities, and in 2009 led to Nobel Prizes awarded to the investigators.

Pharma companies have also found these X-ray facilities extremely useful. Rather than exploring unknown molecular structures, they typically focus on comparing how a number of slightly different drug candidate molecules attach to a cell, seeking those that are most effective in altering the disease process in that cell. In effect, pharma scientists use the X-ray tools to optimize drug candidate molecules before taking them through the expensive process of clinical trials. To do this, pharma companies have partnered with the BES shared research facility at Argonne National Laboratory near Chicago through a unique consortium—the Industrial Macromolecular Crystallography Association (IMCA). This consortium includes five major pharma companies (and another group of temporary members) that are fierce competitors, but which have collaborated for over 20 years to build and maintain X-ray beamlines dedicated to pharma use. In effect, IMCA and a similar consortium using the X-ray source at the Lawrence Berkeley National Laboratory in California share common

research tools. These dedicated beamlines have allowed pharma companies to screen more than 20,000 potential drug candidates a year, resulting in hundreds of potential new drugs now in clinical trials. IMCA reports that about 85 percent of its members' drug development effort is based on access to these high-intensity X-ray tools.

Equally important, however, is the foundational biological research on which pharma companies rely to create their products. One especially pertinent example is the effort by academic scientists to determine the structure of the cellular receptors—known as G-protein coupled receptors—to which many drugs attach. These receptors play a central role in how cells communicate with each other via chemical messengers, translating those messages—as well as sight, smell, and taste messages—into specific actions inside individual cells. In effect, the receptor links with incoming chemical messages, communicates through the cellular membrane, and alters the internal cellular machinery. Understanding how the receptor accomplishes these tasks is key to the rational design of new drugs.

A receptor is itself an extremely complex protein. Scientists first had to obtain pure samples and convert them to crystalline form, then they used the BES X-ray facility at Argonne National Laboratory to analyze the structure.



The structure of a G-protein coupled receptor activating a G protein as revealed by X-ray analysis. *(Brian Kobilka / Stanford University)*

Brian Kobilka (left) and Bob Fischetti (right) discuss how to adjust the X-ray beamline that they used to determine the structure of the complex molecule known as the G-protein coupled receptor. *(Mark Lopez / Argonne National Laboratory)*

Box

HOW SCIENTISTS AND SHARED RESEARCH FACILITY STAFF COLLABORATE TO ADVANCE KNOWLEDGE

The effort to determine the structure of the G-protein coupled receptor faced several challenges. Professor Brian Kobilka, who shared the Nobel Prize for this work, describes the challenge: "It was very hard to grow crystals of the molecule for X-ray analysis, resulting in very small crystals. They could only be grown in an opaque liquid, making the crystals themselves invisible. And the crystals were very sensitive to damage from X-rays. So it initially proved impossible to properly align the crystals with the X-ray beam and collect useful data at the Argonne X-ray facility near Chicago.

"What made the difference is that the X-ray facility scientific and technical staff led by Bob Fischetti and Janet Smith dug in to help, suggesting approaches and making successive improvements to the beamline's capabilities. First they created tools to generate micro-beams that probed the liquid sample with very limited amounts of radiation—identifying the positions and alignments of the crystals. Then they created software controls to allow the beam to home in on the crystals, using very short bursts of X-rays to collect data rapidly before the crystal degraded and other software to synthesize the data into a composite image of the structure. Working together, the tools improved and the process got better and better. We couldn't have determined the structure of the receptor without this collaboration. That's why we included the Argonne team as co-authors on the scientific paper announcing the discovery."

As Bob Fischetti recalls, "We were already working on ways to study small crystals with micro-beams when Brian approached us, but he drove us to redouble our efforts. A key step was a tool that allowed researchers to adjust beam size to match their sample in seconds, as well as improvements in the stability of the beam—all of which have since proved very useful for other researchers. In effect, the challenges that individual researchers bring to us help catalyze continual improvements in our facilities."

Because only very small samples could be obtained, the National Institutes of Health (which supports beamlines for biological research at BES facilities) funded development of ultra-small X-ray beams to facilitate such research (see box). The effort was successful, and the importance of figuring out the structure of the G-protein coupled receptor together with its cellular signaling partner was recognized with a Nobel Prize in 2012. Today, a large proportion of the drug candidates in clinical trials work by attaching to members of this family of receptors, which underscores the impact of both the fundamental structure research and the shared research X-ray tools. It will likely be possible, for example, to have drugs that treat pain, but neither cause dependence nor suppress breathing. And not just opioid users, but people suffering from many now incurable but painful conditions, may benefit.