Hemoglobin is critical to maintaining safe levels of nitric oxide in the body. Above: Hemoglobin alpha (red) seen in a layer of endothelial cells taken from a mouse artery. The blue stains cell nuclei. Opposite page: Hemoglobin alpha (red) and the enzyme nitric oxide synthase (light green) meet up (yellow) in the endothelial cells of a mouse artery.
Found in car exhaust, smog, and cigarette smoke, nitric oxide (NO) was, at one point, mainly seen as a pollutant. In 1992, however, the journal *Science* named NO “Molecule of the Year” for its crucial role in the normal function of the heart, lung, brain, pancreas, uterus, and liver, among many other organs. Six years later, Robert Furchgott, Louis Ignarro, and Ferid Murad were awarded the Nobel Prize in Physiology or Medicine for their discoveries related to NO in the cardiovascular system. (As these scientists pursued the mysterious gas behind heart health, Pitt alum John Hibbs Jr. was describing NO’s biochemical pathway in relation to the immune system.)

As a signaling molecule, or biological go-between, NO helps cells work together. It is produced in the cells that line blood vessels and affects many vital activities, such as blood vessel dilation, inflammation reduction, and blood clot prevention. NO is routinely used in clinical settings. For example, medications that release NO are used to treat high blood pressure and heart failure in adults, and NO gas helps newborns with pulmonary hypertension.

NO can do a body good. But, of course, the gas is also a known danger. An over-abundance of NO can lead to major medical conditions, including septic shock. Striking approximately 750,000 Americans each year, and killing 28–50 percent of those people, according to the National Institute of General Medical Sciences, sepsis is caused when the body produces an overwhelming amount of NO to destroy an infection.

This reaction leads to low blood pressure and blood clotting, and a lack of oxygen can cause organ failure.

With so much at stake, it’s essential to maintain a careful balance of NO in the body. So what keeps it in check? The answer is hemoglobin, reports Adam Straub, a PhD assistant professor of pharmacology and chemical biology and investigator in the University of Pittsburgh’s Heart, Lung, Blood, and Vascular Medicine Institute.

When Straub was a postdoctoral research fellow at the University of Virginia, he was lead author of a paper that showed hemoglobin, the protein in red blood cells that carries oxygen, controls the diffusion and signaling of NO in the blood vessel wall. The paper was published in November 2012 in *Nature*.

“We used to think that NO just went everywhere in the body, but it’s actually highly regulated,” says Straub. Simply put, hemoglobin controls NO by deactivating it. The process, Straub explains, is similar to that of a garden hose; hemoglobin acts like a nozzle, regulating the amount of NO and how much blood vessels dilate.

What’s more, researchers are also finding hemoglobin in cell types other than red blood cells, including in the brain, lungs, and kidneys.

“This shows a much wider impact in the body in terms of how NO affects many functions, such as how neurons talk to each other in the brain and how immune cells talk to each other about inflammation,” says Straub.

Today, Straub’s work adds to a long and thriving legacy of NO research at Pitt. Those investigations have ranged from identifying the human gene that ultimately triggers NO production, to understanding how the molecule contributes to organ transplant rejection, to studying how to increase NO levels in order to treat heart and lung problems.

To that end, Straub’s latest research involves the development of peptides that disrupt hemoglobin’s NO-deactivation mechanism. The result is a decrease in blood pressure and possibly even a complete reversal of hypertension. So far, animal models have shown promising outcomes in this area of study, and Straub is exploring the next steps for future inquiry.
It is sometimes surprising to be reminded that the law of parsimony almost always applies—even in a field as intricate as neurodegenerative disease. But, as a University of Pittsburgh team reported in Nature Neuroscience this May, Huntington’s disease (HD), a protracted and “universally fatal” neurodegenerative disorder, is actually caused by a case of clogged cellular plumbing.

More than 30,000 Americans suffer from HD—an untreatable condition. Symptoms of the disease often emerge in mid or even late life, after which patients may live another 15 years or so. Most of the damage is to the brain—the cortex and striatum—where neurons die slowly. Because neurons rarely regenerate, the effects of HD accumulate and escalate over a lifetime; hallmark symptoms range from involuntary muscle movement to cognitive and psychological dysfunction.

Researchers have known that HD is associated with a mutation influencing the production of huntingtin protein (HTT). They also knew that deaths of neurons in the brain cause the devastating symptoms of the disease.

Pitt’s Robert Friedlander, who is chair and Walter E. Dandy Professor of Neurological Surgery, and colleagues wanted to establish how the mutant protein huntingtin actually caused these cells to die. It involved years of painstaking investigations, but they figured it out.

It all comes down to the mighty mitochondria—which produce the power that fuels all cellular function.

Mitochondria need some 1,200 different proteins to generate all this energy. Most of those are assembled in the cell’s cytosol and imported through protein channels in the mitochondrial membrane. Without a full complement of functional proteins, mitochondria cannot operate properly.

Friedlander and his colleagues suggested that mutant HTT might have a proclivity for binding to protein import channels—passageways in the mitochondrial membrane through which proteins emigrate from the fluid inside of cells. Mitochondrial dysfunction and cell death in HD might be explained, they thought, if mutant HTT were shown to block functional proteins from getting inside the mitochondria.

Using neurons from the brains of both humans and mice with HD, the researchers were able to follow the problem upstream to its source—an import channel defect. In particular, mutant HTT binds with all three of the proteins that make up a channel called TIM23. Once clogged by mutant HTT, TIM23 can no longer import critical functional proteins, and the cell gradually weakens and dies.

Elizabeth Jonas, an MD on Yale University’s medicine and neurobiology faculty, wrote a study review of the paper in Nature Neuroscience; she noted later in an interview that the Pitt work is “thought-provoking.”

“These findings will definitely be of interest to anyone in the field of HD, but also to people working on any problem that involves aggregations of abnormal proteins that could block channels,” Jonas says.

“Friedlander’s team found that synaptic mitochondria are more susceptible [to clogged plumbing in HD],” she adds. “That was tremendously exciting. ... Why doesn’t the disease manifest until so late in life? It must mean that only a few of the many mitochondria are affected at first—and that slows down the synapses just enough to make the patient not able to run track or answer a question on an exam. Over the course of the patient’s life, the synapses are taking more and more hits, and those hits are accumulating until finally they reach some threshold, and we see the full clinical manifestations.”

Friedlander’s team also wondered, Could we protect neurons by boosting levels of the three proteins that make up TIM23? It wasn’t easy to find out. Says Friedlander, “it is very, very difficult to transfect the three proteins that make up the TIM23 complex, especially into the primary neurons”—the first casualties of HD.

This phase of the study took five years.

It turns out that overexpressing the proteins that make up TIM23 can “rescue” neurons subjected to mutant HTT—unprecedented findings that were well worth the wait.
Preeclampsia is a puzzle to medical researchers—no one knows what causes it. Characterized by high blood pressure and protein in the urine of pregnant women, preeclampsia causes seizures, disability, and even maternal or infant death. The only cure is delivery, which can mean a premature induction or cesarean section if a mother’s symptoms are severe.

Lisa Bodnar (Fel ’04), an MPH/PhD assistant professor of epidemiology at the University of Pittsburgh Graduate School of Public Health and of obstetrics, gynecology, and reproductive sciences as well as psychiatry at the School of Medicine, notes that preeclampsia, which affects 3.4 percent of pregnant women in the United States, accounts for 18 percent of maternal deaths associated with births each year.

Bodnar, a trained dietitian, has been interested in pregnancy since she was an undergraduate at the University of North Carolina at Chapel Hill, where she examined folate levels in pregnant women. She has been intrigued by the effects of diet and nutrition on gestation ever since.

When Bodnar returned to her native Pittsburgh for a reproductive biology postdoctoral fellowship at Magee-Womens Research Institute, where she’s now on the faculty, she began researching pregnancy and vitamin D, a unique nutrient in that we both absorb it from our food and synthesize it in our skin following exposure to the sun.

“People have always related vitamin D to bone health and calcium metabolism,” she says. But about a decade ago, the literature began to suggest it could also be important for other reasons, including placental function and regulating placental inflammation. “I felt like preeclampsia was a really obvious direction to take the research from there.”

She and her team recently examined vitamin D levels in blood samples from pregnant women collected as part of the Collaborative Perinatal Project (CPP)—a 12-center study conducted from 1959 to 1966. Bodnar’s investigation focused on samples gathered in the first 26 weeks of gestation, roughly 700 being from women who later developed preeclampsia and about 3,000 from women who did not. The samples were well preserved and able to be tested for vitamin D levels four decades later.

Bodnar’s team controlled for factors that can influence vitamin D status—including smoking, diet, ethnicity, and physical activity—and found a correlation between vitamin D deficiency and the rate of severe preeclampsia. In fact, sufficient vitamin D intake was associated with a 40 percent reduction in the incidence of severe preeclampsia. Supports a correlation between vitamin D and preeclampsia—not causation. Her ongoing research explores how the body metabolizes vitamin D to better understand the link between this deficiency and adverse pregnancy outcomes. She believes vitamin D will prove to be a key component of a healthy pregnancy.

In October 2013, the American Journal of Epidemiology published another paper by Bodnar’s group examining ethnicity and preterm birth. In CPP participants of African American and Puerto Rican descent (populations that are more susceptible to vitamin D deficiency than whites), Bodnar’s team found that incidences of premature birth decreased by as much as 30 percent as vitamin D levels increased. In white women, the team found no such relationship between vitamin D and preterm birth. The researchers also found that vitamin D deficiency was most strongly associated with cases of preterm birth involving placental damage resulting from inflammation.

What excites Bodnar most about her work is its potential for a wide impact, should the causal relationship pan out in other studies now under way.

“Even if [vitamin D deficiency] is only related to a small subset of adverse outcomes, the treatment is so simple, so inexpensive, and so safe,” she adds.

“Vitamin D and other nutrient deficiencies are attractive to study because they are modifiable.”