Fluorescent biosensors light up high-throughput metabolic engineering

Genetically encoded fluorescent biosensors allow researchers to see how products form in real time in microorganisms, and to test billions of candidates at a time

Synthetic biologists are learning to turn microbes and unicellular organisms into highly productive factories by re-engineering their metabolism to produce valued commodities such as fine chemicals, therapeutics and biofuels. To speed up identification of the most efficient producers, researchers describe new approaches to this process and demonstrate how genetically encoded fluorescent biosensors can enable the generation and testing of billions of individual variants of a metabolic pathway in record time.

Biotechnologists that tinker with the metabolism of microorganisms to produce valued products look at the engineering process through the lens of the so-called 'design-build-test cycle.' The idea is that multiple iterations of this cycle ultimately allow the identification of combinations of genetic and metabolic elements that produce the highest levels of a desired drug or chemical. Key to the cycle's efficiency, however, is the ability to construct and test the largest number of variants possible; in the end, only a few of these variants will produce the product in industrially attractive amounts.

Bioengineers thoroughly understand how metabolic pathways work on the biochemical level and have a plethora of DNA sequences encoding variants of all of the necessary enzymes at their disposal. Deploying these sequences with the help of computational tools and regulating their expression with an ever-growing number of genetic elements, gives them access to an almost infinite pool of design possibilities. Similarly, revolutionary advances in technologies enabling DNA synthesis and manipulation have made the construction of billions of microorganisms, each containing a distinct design variant, a routine process.

Source: www.sciencedaily.com

-Contributed by Krishna Saraiya IGBT IV , Dipika Patel

From prediction to reality: a history of the search for gravitational waves

- **1915 -** Albert Einstein publishes general theory of relativity, explains gravity as the warping of spacetime by mass or energy
- **1916 -** Einstein predicts massive objects whirling in certain ways will cause spacetime ripples—gravitational waves
- **1936 -** Einstein has second thoughts and argues in a manuscript that the waves don't exist—until reviewer points out a mistake
- **1962 -** Russian physicists M. E. Gertsenshtein and V. I. Pustovoit publish paper sketch optical method for detecting gravitational
- waves—to no notice
- **1969 -** Physicist Joseph Weber claims gravitational wave detection using massive aluminum cylinders—replication efforts fail
- **1972 -** Rainer Weiss of the Massachusetts

Institute of Technology (MIT) in Cambridge independently proposes optical method for detecting waves

- **1974 -** Astronomers discover pulsar orbiting a neutron star that appears to be slowing down due to gravitational radiation—work that later earns them a Nobel Prize
- **1979 -** National Science Foundation (NSF) funds California Institute of Technology in Pasadena and MIT to develop design for LIGO
- **1990 -** NSF agrees to fund $250 million LIGO experiment
- **1992 -** Sites in Washington and Louisiana selected for LIGO facilities; construction starts 2 years later
- **1995 -** Construction starts on GEO600 gravitational wave detector in Germany, which partners with LIGO and starts taking data in 2002
- **1996 -** Construction starts on VIRGO gravitational wave detector in Italy, which starts taking data in 2007
- **2002–2010 -** Runs of initial LIGO—no detection of gravitational waves
- **2007 -** LIGO and VIRGO teams agree to share data, forming a single global network of gravitational wave detectors
- **2010–2015 -** $205 million upgrade of LIGO detectors
- **2015 -** Advanced LIGO begins initial detection runs in September
- **2016 -** On 11 February, NSF and LIGO team announce successful detection of gravitational waves.

Physicists working with the Laser Interferometer Gravitational-Wave Observatory (LIGO) announced that after decades of effort they had detected gravitational waves—ripples in spacetime itself—set off by the explosive collision of two massive black holes. But which of the 1000 scientists who work on LIGO, a pair of gargantuan instruments, was the first to see the long-awaited signal?

His tale shows how elaborate plans devised to keep LIGO team members guessing whether a signal is real or a purposefully planted fake broke down, leaving one lucky physicist and, soon, the entire LIGO collaboration sitting on a thrilling secret.

Marco Drago wasn't in Louisiana or Washington, or even the United States. Instead, the 33-year-old postdoc from Padua, Italy, was at his office at the Max Planck Institute for Gravitational Physics in Hanover, Germany, where members of the LIGO team work on data analysis. There, Drago oversees one of four data "pipelines," automated computer systems that comb through the raw data coming out of the two detectors looking for potentially interesting signals. On 14 September 2015, while Drago was on the phone with a LIGO colleague in Italy, his pipeline sent him an email alert—of which he receives about one each day—telling him that both LIGO detectors had registered an "event" (a non-routine reading) 3 minutes earlier, at 11:50:45 a.m. local time. It was a big one. "The signal-to-noise ratio was quite high—24 as opposed to [the more typical] 10,".In fact, the signal was so strong that Drago didn't believe it was real—and with good reason. A gravitational wave from a distance source stretches space by an infinitesimal amount, and to detect that rhythmic stretching LIGO employs two gigantic optical devices called interferometers, which essentially act as gigantic rulers. To test the incredibly complicated devices, LIGO physicists have developed mechanical systems to give them a shake and "inject" a fake signal.

The signal Drago saw was so perfect it seemed too good to be true, he says. “No one was expecting something so huge, so I was assuming that it was an injection.”

Injections can be done in two ways: out in the open when researchers are tuning up the machines and secretly when they are taking data. Those latter “blind injections” are meant to keep researchers on their toes. Only four LIGO leaders know when such injections are made, and that information is supposed to be revealed only after a potential signal has been thoroughly scrutinized and written up for publication. That’s how things unfolded in 2010, when LIGO researchers learned at the last minute that a possible signal was in fact a blind injection. So if all had gone as anticipated, Drago might have simply noted the alert and carried on as usual, assuming the truth would come out in the end. Drago knew that the injection system was not supposed to be working. He immediately set out to verify that and ended up alerting the entire collaboration to the signal.

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Using light to control protein transport from cell nucleus

Light can be used to control the transport of proteins from the cell nucleus with the aid of a light-sensitive, genetically modified plant protein. Biologists working in the field of optogenetics have now developed such a tool. The researchers employed methods from synthetic biology and combined a light sensor from the oat plant with a transport signal. This makes it possible to use external light to precisely control the location and hence the activity of proteins in mammalian cells.

Eukaryotic cells are characterised by the spatial separation between the cell nucleus and the rest of the cell. "This subdivision protects the mechanisms involved in copying and reading genetic information from disruptions caused by other cellular processes such as protein synthesis or energy production," explains Prof. Eils, Director of Heidelberg University's BioQuant Centre and head of the Bioinformatics Department at Ruperto Carola and the DKFZ. Proteins and other macromolecules pass through the nuclear pore complex into and out of the cell nucleus in order to control a number of biological processes.

While smaller proteins passively diffuse through the nuclear pores, larger particles must latch onto so-called carrier proteins to make the trip. Usually short peptides on the protein surface signal the carriers that the protein is ready for transport. This signal is known as the nuclear localization signal (NLS) for transport into the nucleus, and the nuclear export sequence (NES) for transport out of the nucleus. Artificially inducing the import or export of selected proteins would allow us to control their activities in the living cell. The Di Ventura lab has specialised in optogenetics, a relatively new field of research in synthetic biology. Optogenetics combines the methods of optics and genetics with the goal of using light to turn certain functions in living cells on and off. To this end, light-sensitive proteins are genetically modified and then introduced into specific target cells, making it possible to control their behaviour using light. The hybrid LOV2-NES protein can be attached to any cellular protein and used to control its export from the nucleus using light.