Engineering jellyfish protein to develop molecular probes: Aequotech (Ferrara, Italy)

The Jellyfish's Shiny Interior

Just three years after its foundation, a start up from a small medieval town in Italy has taken its first steps to success. Generating new molecularly engineered sensors of biological parameters, Aequotech is to automate them for drug screening and similar applications.

Your *Lab Times* reporter still remembers the day, in summertime, when she was taking a walk on the shores of the Mediterranean. Very soon the heat became too much to bear and she decided that she really needed to take a plunge in the green sea. She drifted away from the beach, swimming, and everything would have been perfect had something not stung her leg. It was a sharp pain on the left calf and she could no longer move. Fortunately, she was not too far out and could easily reach the shore. A big splash of red erupted over her leg and a sailor, working nearby, looked at it and said, "That's a jellyfish bite. They come near the shore when it's hot. You should be more careful".

Using jellyfish as a source of dye

This was your *Lab Times* reporter's first encounter with a jellyfish. That day, looking at those jelly bubbles moving randomly in the sparkling water, she couldn't imagine that decades before in a faraway laboratory in the north west of the United States, someone had found a way of using jellyfish to move molecular biology towards new frontiers and applications. It was 1961 when Osamu Shimomura at the Friday Harbour Laboratory began to study the bioluminescence of the jellyfish *Aequorea victoria*, leading to the use of two jellyfish proteins, aequorin and GFP (green fluorescent protein), in all sorts of biological research.

Let's fast forward to 2009. When visiting Ferrara, a small medieval city in the north east of Italy, your reporter again crossed paths with her jellyfish nemesis. For this is where the biotech company Aequotech was founded in 2005, as a spin-off of the University of Ferrara, by five experts in calcium signal dynamics: Tullio Pozzan, Rosario Rizzuto, Paolo Pinton, Carlotta Giorgi and Marisa Brini. The Aequotech laboratory is based on a university campus, in a flowered garden surrounded by the city walls. It is not easily reached by car if one doesn't know the city and all its narrow streets.

Aequotech founder Paolo Pinto (in the middle, with glasses) and his team of protein experts are developing new fluorescent proteins from the jellyfish *Aequorea victoria*.

Photo: Aequotech

Investigating fluorescent proteins in medieval Italy

What has Aequotech got to do with jellyfish? The 'meeting-point' is that the company specialises in engineering the jellyfish aequorin protein to develop new molecular probes and reporters for medical treatments. Aequorin from the *Aequorea victoria* jellyfish is a bioluminescent protein, composed of a protein portion (apoprotein) and a prosthetic group (coelenterazin). These two components combine spontaneously, forming the functional aequorin protein. When the aequorin meets calcium ions, the apoprotein and the prosthetic group dissociate to release a photon of light. The light released by the aequorin can be measured and used as an indication of the calcium ion content inside the cell. Based on this relatively simple biological principle, Aequotech started its walk into the world of pharmaceutical companies.

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"The decisive moment for the Aequotech foundation was in 2003, when we won the Padova Startcup, a competition aimed at finding and funding new ideas that can give rise to small companies in the surrounding area," begins Paolo Pinton, one of the founders. "Then we decided to establish the Aequotech in Ferrara," he continues, "because this city, with its open-minded cultural environment, gave us the right place where we could begin this new experience".

A key figure for the company was Carlotta Giorgi, a young researcher who decided to be the spin-off's link between the lab's research and its applications. Pinton happily explains, "In Aequotech there are two distinct realities. The research we make in the lab and the work we make in the spin-off". In fact, "It is a primary necessity for us that the spin-off activity and the research activity remain separated. Research for definition is the trying of new ideas and their test. The spin-off takes from the research what can be useful for the market and works to get a product. What leads is the research that has not to be 'contaminated' by the market demand. This has been possible only thanks to the work that Carlotta Giorgi has done".

Targeting fusion proteins to cells' compartments

But, since the cloning of aequorin on a plasmid vector and its expression in cell lines had already been performed by other research groups, a spontaneous question arises: What's so sensationally new about Aequotech?

Pinton can answer this question, emphasising that, "We have been the first to perform the targeting of aequorin based fusion proteins to different cellular compartments, for the screening of new drugs". In particular, despite its short history, Aequotech has already patented two products. When asked to explain in detail the subject of the two patents, Pinton holds a pen in one hand and a sheet of paper in the other and starts to sketch a cell and its components.

"The first patent concerns the translocation of aequorin-based fusion proteins to the cell membrane. The cell cytoplasm has a low content of calcium ions if compared with the sub-cellular micro-environment under the cell membrane. So, we designed different

The bioluminescent jellyfish *Aequorea victoria*. In this organism, Nobel laureate Osamu Shimomura discovered the green fluorescent protein (GFP), developing it to an important biological research tool.

kinds of fusion proteins that contain the aequorin sequence linked to a particular case study protein. For example, one protein we are studying now – let's call it protein X – is responsible for the signal transduction from a membrane receptor to the cytoplasm. When over expressed by the cell, X leads to the diabetes phenotype development. For this reason, it is important to find an experimental protocol that could allow the screening of different drugs directed against it in order to prevent the diabetes arising."

Testing drugs via light emission

Pinton continues, "The hybrid protein by Aequotech, once it has been produced by the cell, mimics the natural one and reaches the cell membrane to contact the receptor. In that moment, a release of light occurs from the aequorin portion in response to the variation in calcium concentration. Knowing this, it is possible to test different drugs directed against the specific protein by measuring the emission of light from the aequorin fusion protein after drug treatment. Light release will be proportional to the amount of functional proteins that reach the membrane and this will be an indication of the effectiveness of the tested drug in contacting and interacting with the target molecule".

The second patent concerns the use of a different bioluminescent protein. In particular, a luciferase-fusion protein is targeted to the cell membrane in the presence of the prosthetic group luciferin. The mechanism is similar to that of the aequorin-coelenterazin: in the presence of ATP, luciferase and luciferin dissociate to yield light emission. The emitted signal is once again proportional to the amount of ATP molecules released from the cell. This system is useful for testing and screening the toxic effect of new biomolecules expressed as ATP released after drug treatment.

Prepared for automation

After our conversation, Pinton shows your *Lab Times* reporter around the Aequotech laboratory and facilities. What is immediately striking is a big robot enclosed inside the cell fan.

"That is the electronic platform for experiment automation," says Pinton, noticing the reporter's interest, "Everything we want to sell as a company has to be ready and suitable for automation. This robot can take away the medium from cell cultures or seed the cells in different plates. It helps us in the every day research, because it can handle the cultures and the drug treatments independently. But, moreover, it allows us to test our protocols for their automation."

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Speaking about his work of a lifetime, Pinton underlines, "The use of luminescent proteins has a lot of advantages. In fact, luminescence is a 'clean and clear' signal to be measured that has not all the noisy interferences from the cell environment that fluorescence, for example, has."

From the outside, this would seem to be an example of company puff but, taking a look at the Aequotech webpage, it is at least partly substantiated. Among Aequotech's clients are a lot of big names: the Center for Human Genetic Research (Massachusetts General Hospital), the Harvard Medical School's Department of Medicine (both Boston,USA), the Charité Biomedical Research Center (Berlin, Germany), the Insect Molecular Genetics and Biotechnology Institute and the National Centre for Scientific Research Demokritos (Athens, Greece), and the Suven Life Sciences (Hyderabad, India). Can little Aequotech really boast all these organisations as customers?

Big names on the customer list

When asked about this, Pinton affirms that the aequorin-based expression systems developed by his company have already been purchased by these laboratories. They have been, "used with success" for the screening and testing of potential new drugs. "For example," he adds, "Tomorrow I am going to Boston and New York just to meet our American clients".

Listening to Pinton's stories and plans takes you back to the days of graduation and leaving university looking for work. The faculties' improbable advice to students only served to reinforce their ivory-tower stereotype. In other words, irrelevant nonsense. In reality, a better route to success is to be confident in the potential of one's own ideas (which, admittedly, sounds trite), however, "aequorin company" is proof that hackneyed phrases at least sometimes hold true.

At the end of the conversation, Paolo Pinton points out that for every spin-off company, the first years are decisive. In his case, "The coming year will be crucial for defining the future of Aequotech."

By the way, Osamu Shimomura's lengthy liaison with jellyfish was also successful. In 2008, the Japanese marine biologist was awarded the Nobel Prize in Chemistry for the discovery and development of green fluorescent protein (GFP), together with two American colleagues.

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