in vivo fiat lux

WatchFrog

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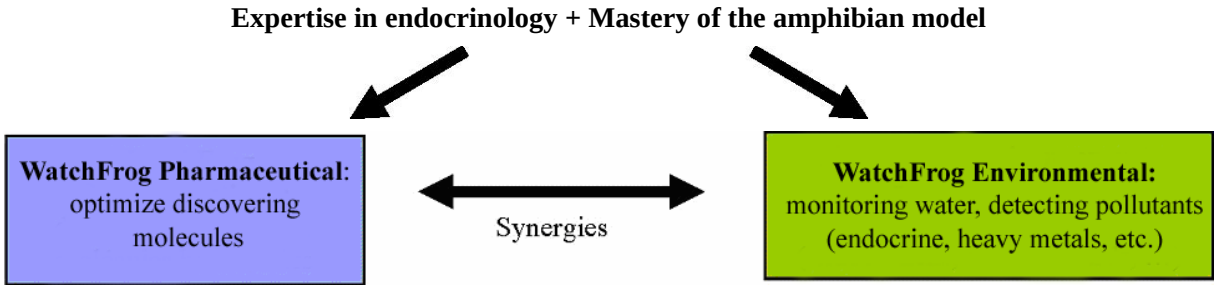
WatchFrog business profile

WatchFrog creates and markets innovative solutions for the *in vivo* detection of a wide range of chemical, pharmaceutical and cosmetic substances. We provide pharmaceutical companies with a unique methodology for developing new molecules. Our genome-based technology incorporates high biological significance while substantially reducing drug development costs.

WatchFrog's versatile technology derives from a fundamental research program carried out within an internationally recognized research laboratory affiliated to the CNRS (National Research Council) and located within the French Natural History Museum.

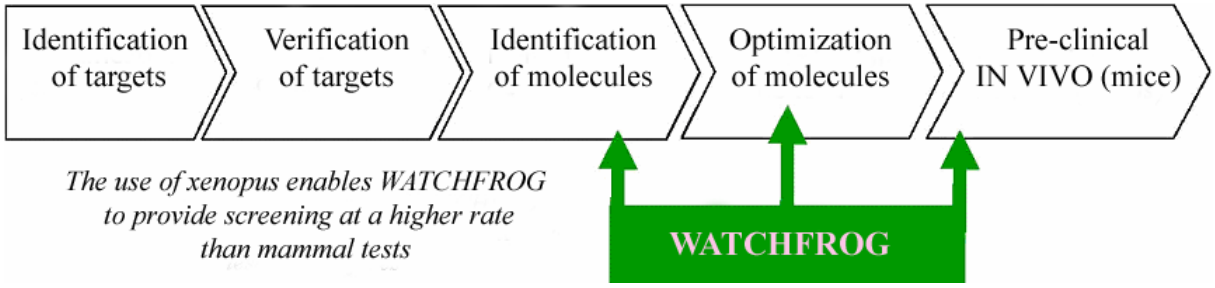
WatchFrog technology is applicable to two main areas: the environmental market and the pharmaceutical market, as schematized below.



WatchFrog Pharmaceutical

Following the *in vitro* screening stages, WatchFrog technology will enable high potential molecules to be selected with precision, rapidity and low cost, prior to the implementation of further and higher-cost development using mammals.

WatchFrog technology thus comes into operation as a bridge between *in vitro* screening and the pre-clinical stages.



The primary advantage of developing therapeutic models with xenopus is that these tests will be more economical and faster than traditional models using mammals.

The genetic and physiological proximity between humans and xenopus is particularly well established and recognized for endocrine regulation. Hence diseases such as osteoporosis and certain hormone-dependent cancers for which hormonal treatments have been developed are applications particularly well-suited to our models.

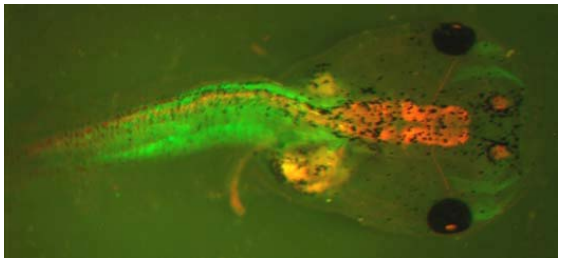
Other physiological and pathological fields can also benefit from our technology. For these, xenopus is again relevant in that it very rapidly develops a vascular system and a complex central nervous system in the course of its growth. Thus we are able to develop target-models to test new molecules of angiogenic or neurological interest.

More particularly, as far as neuropathologies are concerned, our ancillary know-how in gene transfer in the mammal brain, enables us to offer a second level of expertise for neuroprotector drugs.

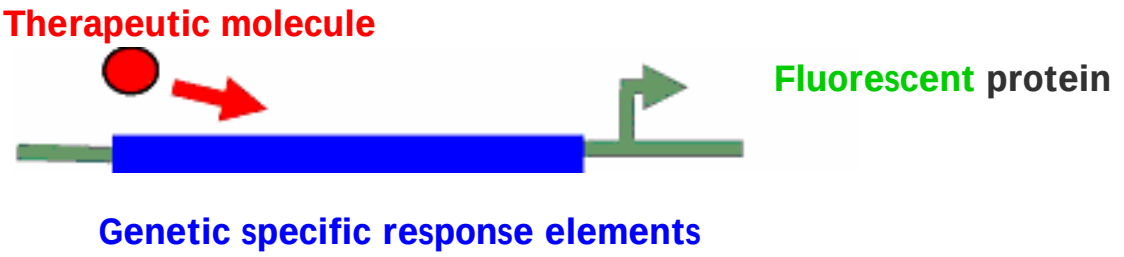
Yet another advantage of the xenopus model is that it has exceptionally large pigmentary cells (melanocytes) on its skin. This feature makes xenopus particularly attractive for the observation of this cell type, of which deregulation of the cycle is the cause of melanomas. WatchFrog technology in association with this characteristic could open the way to the development of models of great potential for this pathology.

The new generation of tests offered by WatchFrog is allied to the development of amphibian models that “light up” (through emission of fluorescence) when a biological function is activated.

Technology: WatchFrog technology is covered by a number of patents.



Larvae light up in response to a molecule activating one or more biological functions. Various luminous signals (in the image shown, red and green) enable the specificity of action in relation to an organ to be ascertained (here, nerve tissue red and muscles green).Our system enables this activation to be located and quantified *in vivo* on the pertinent and easy-to-use xenopus model.



The basic principle involves creating genetic constructions that enable a GFP (Green Fluorescent Protein) to be expressed in response to the physiological action of whatever type of molecules our customers may be interested in. This “genetic detector” is then incorporated a xenopus larva, thereby taking into account all the biochemical regulations that can respond *in vivo* to the sample being tested.

For example, the effect on a physiological function such as osteogenesis of a hormonal substance is revealed by the activation of response elements to the hormones specific to bone regulations, triggering the synthesis of fluorescent proteins. The fluorescence is visible through the transparency of the organism, and can therefore be detected and quantified without sacrificing the animal. The larvae simply need to be placed in the liquid sample to implement the test. The genetic constructions can be altered as required to produce a tailor-made range of tests to respond to various disruptive or pharmacological effects.

This test methodology combines the advantages of ***in vivo*** with the flexibility of ***in vitro.*** It rapidly and simply furnishes accurate information of high sensitivity and specificity, together with low cost, economic use of material, and the potential for automatization.

In cases of contact or absorption, the physiological action of a product/substance on the organism will modify the expression of the genes relating to this effect. The mechanism brings into play genetic elements which are activated in the presence of the substances to be detected.

WatchFrog technology is **innovative** in several respects:

. • Detection at the genetic level or **“geno-detection”** of the *in vivo* distribution of pharmacological molecules. This allows a rapid response, since the expression of a gene in response to a signal is detectable after a few hours, while the assessment of biological consequences can require several days. What’s more, this integrates all the biochemical regulations that precede the activation of the genetic system while remaining specific to a given function.

. • The **choice of species** as the basis for the tests. Xenopus has denser and more rigid bones than fish, as well as a more complex heart and circulatory system. In addition, in terms of endocrine

physiology, the conservation of biochemical mechanisms between xenopus and humans has been demonstrated and proved. Xenopus is an investigated and recognized model in the research world, but its industrial potential has yet to be fully exploited.

. • The method of **rapid *in vivo* selection** of genetic constructions, which brings considerable time savings and offers great flexibility for creating a battery of tailor-made tests.

. • The **automatization of reading results *in vivo***, which allows the simplicity and low cost of *in vitro* tests to be combined with the biological pertinence of *in vivo* analysis.

. • Mastering **the techniques of breeding amphibians** in large numbers. This variously involves ensuring perfect sanitary conditions, obtaining high reproduction levels, and maintaining an environment suited to the rapid growth of the animals.

Competitive advantages

In vitro tests have little predictive power and conventional *in vivo* tests are cumbersome and expensive to implement. WatchFrog's competitors consist mainly of US and European companies which have developed other animal models (such as worms and fish) that are less close to humans than amphibians are (amphibians having the advantage of 150 million years evolution over fish).

WatchFrog amphibian tests have a number of technical advantages similar to those of ***in vitro*** tests:

- **simplicity of administering** the substance to the animal, by simply dissolving the substance in water, and direct absorption
- **compatibility** with standard equipment through the use of 96-well micro-plates
- **small quantity of active substance** needed (tests in micro-wells)
- **high statistical validity** of results at low cost, due to the very large number of genetically identical eggs in each laying
- **rapidity** of screening, with fluorescence detectable after a few hours
- **ease of reading** results by measuring fluorescence via transparency, without sacrificing the animal or performing an autopsy
- **sensitivity** from concentrations of 10^{-11} molar upward
- **quantification of the effect** by measuring the dose-response relation on a fluorimeter
- minimization of **ethical** issues by virtue of using amphibian larvae (which are not considered to be animals)
- **low cost** of maintenance and production of amphibians in comparison with mammals.

WatchFrog amphibian tests also have various technical advantages similar to those of ***in vivo*** tests:

- an **integrated approach** (on a vertebrate) that enables the test to have a **biological significance** by taking into account the regulating effects of the complete metabolism and the distribution of the molecule in the organism
- the use of the xenopus **model** gives results recognized as being similar to those of mammals and humans, in particular for the endocrine, angiogenic and neurogenic systems
- **poly-detection** of a number of metabolic functions and genes simultaneously through the use of multiple markers and fluorochromes
- the possibility of producing ubiquitous and/or tissue-specific constructions

Our location and development within the Natural History Museum/CNRS conforms to the standards for experimental analysis demanded by a leading cosmetics company.

WatchFrog enables the potential of new molecules to be tested rapidly and *in vivo* while integrating one or several targets. These targets can be specific to a signaling pathway, to a physiological/pathological effect, or to a cell type. Already applying its technology for environmental risk assessment (with Electricité de France), WatchFrog is looking for an innovative pharmaceutical partner.