Are you a chassis?

A philosophical investigation into the introduction of *Xenopus tropicalis* as a new chassis in the iGEM contest

By

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"Regarding the service done to science, frogs deserve the first place. No animal ever serve more numerous and greater discoveries on every aspects of science, and still today, without frogs, physiology would be impossible. If the frog, as one said, is the Job of experimental physiology, that is to say the most maltreated animal by the experimenter, it is undoubtedly the animal most closely associated with their labor and scientific glory." Claude Bernard, 1865 *Introduction à l'étude de la médecine expérimentale*, Deuxième partie, chap. II, VI

Hi Xenopus*!*

The starting point of our investigation can be summed up in one sentence: "*Xenopus tropicalis* is introduced as new chassis in the iGEM contest". This introduction appeared to the Evry team members raising important "ethical and maybe legal issues" that should involve the help of a philosopher (or maybe someone else from the humanities). The "need of ethics" is a feeling quite difficult to characterize, we have the idea that something is at stake in what we do but we can't really formulate what or why. But a tension exists, might exist or should exist, somewhere in the lab or with society. Our sentence, "*Xenopus tropicalis* is introduced as new chassis in the iGEM contest" lead us spontaneously toward very difficult issues concerning animal experimentations and GMOs, tricky topics which often bring sterile debates between pros and contras and other caveats that we don't want to fall into.

It is important to note that the philosopher brought into the team hadn't studied moral philosophy or animal ethics before joining the team. In that respect he was in formation, as the other team members, trying to understand what could be experimental philosophy or some kind of applied philosophy which sounded quite oxymoronic. Problematizing the human practice needed a lot of discussions and debates, as biologists and modelers didn't really know what they were expecting from the philosopher, and the philosopher didn't really understand what "human practice" or the "need of ethics" was. After all, if the problem was the legality of the experimentation or drawing the red line of "when does science stop and cruelty towards animals begins" with tadpoles and frogs, it was not a philosopher that was needed but an expert from an ethical committee stamping "everything is ethically acceptable, you can proceed to the experimentation".

Thus, after we made sure that everything planned was ethically acceptable from the point of view of laws, we tried to understand what should be a "human practice project" dealing with *Xenopus tropicalis*. The main point of those discussions was the following, our human practice should not be about convincing people that what we are doing is great, nor posing the basis of a new start-up using tadpoles for various purposes (it already exists), nor building scenarios on the propagation of our tadpoles outside the laboratory, nor trying to rethink categories used by laws on animal experimentation such as "should we extend or restraint the species concerned by the animal category?" or "when does the larvae should be considered as an animal?" etc. We didn't have time and experience to tackle such issues, we wanted a human practice dealing with our actual work in the laboratory – to the extent of the possibility can be embedded in this practical work.

What should our human practice be then? Some kind of a road book, the witness of a self-reflection triggered in the team during May/June 2012 on our practice, concepts and disagreements, on the paths we could open or close for synthetic biology and iGEM contest. The human practice should also be at the interface of the laboratory and society, articulating each other's expectations and fears. What we deliver here is the organized outcome of the

various discussions and debates we had during the summer, the compromises we tried to build and the issues which stay vivid. *A posteriori* we noticed that those discussions mainly dealt with the sentence previously quoted, "*Xenopus tropicalis* is introduced as new chassis in the iGEM contest", and especially around the terms of "*chassis*" and "genetically *engineered machine*", which specially caught the attention of traditional biologists and laymen when referring to an animal. These terms appeared to symbolize the divergence of perceptions and ethical sensibilities we had among the team. In our account of this summer investigation we developed four aspects of the introduction of *Xenopus tropicalis* in the iGEM contest, aiming at clarifying the range of the engineering metaphors and attitudes applied to living things. The four aspects are the following:

Though these aspects are all related, we tried make the parties readable independently from one another, to give the possibility for one another to start with the part he feels the most (or the less) concerned.

A fifth part, more independent of the rest of the work, is an annex summering the legal aspects of animal experimentation, the debates in La Paillasse and the two surveys which helped us start our investigation.

Last but not least, we have to precise that the story is sometimes biased by the opinions of the writer (the philosopher). Though we try reflecting all the opinions of the team members and insisting on the divergences, some conclusions, and the organization of this work, might not be the fruit of a successful compromise.

Would you be my chassis?

Introducing *Xenopus tropicalis* as a new chassis in the iGEM contest requires a few epistemological investigation. The term "chassis" refers to a specific kind of model organisms which are bound to the specific ways of experimenting developed in synthetic biology. Examples through history have shown that the organisms used for conducting the experimentation often influence deeply the theories and projects inferred. As the term "chassis" is at the center of our study, it is important to remind why *Xenopus tropicalis* is an interesting model organism, and to what extent the term chassis is epistemologically relevant when referring to vertebrates.

Xenopus tropicalis *as a good model organism*

In experimental biology the choice of the right organism is a crucial beginning of research, this has been widely repeated, so was saying Claude Bernard, father of experimental medicine, and confirmed on the matter by many historian and philosophers of biology, such as Lederman and Burian[1]. Though the choice the organism studied is often a matter of contingent circumstances (and it was indeed the case for our research), it often has an undeniable impact on the development of a research. As R. Burian explains, "most biologists realize that the choice of an organism can greatly affect the outcome of well-defined experiments and can thus have a major impact on the valuation of biological theory"[2], thus some model organisms, like *Drosophila melanogaster* and *Escherichia coli* accomplished their role with great success, while others, like "*Hieracium*, *Oenothera* and *Ascaris* […] led investigators astray"[3]. The history of *Drosophila melanogaster* is quite interesting in that matter, as the fly studied by Thomas H. Morgan had a huge impact on the practices and problems of genetics[4], the simplicity of its genome favoring the theory of the central dogma, on gene, one protein, one feature, and the projects of mapping genomes. As genetic modifications had huge impacts on *Drosophila melanogaster*'s phenotype, we put in the genome an explanatory power that neglected important aspects of the complexity of genetic regulation and organisms' environment. However it was a fertile way to limit this ungraspable complexity in a first time.

Synthetic biology brought a new word when referring to model organism: the term "chassis". Though this term may surprise when first heard (one spontaneously think of mechanics rather than biology) it is quite coherent with the project of synthetic biology: making biology easier to engineer. The epistemology of synthetic biology is not a descriptive one but a pragmatic or a technological one. We have to produce knowledge by building and standardizing our construction, to make it possible to work in a large scale. As synthetic biology introduces new terms, it is important to analyze them in order to get clearly what they mean, and try to

[1] A classical reference on that subject is Lederman M. and Burain M.S. eds. 1993 *The right organism for the job*, Vol 26 (2)
[2] Burian R. 2005, *The epistemology of development, evolution and genetics*, New York, Cambridge University Press, p12
[3] *Ibid.* p12
[4] Kohler R.E., 1994, *Lords of the Fly:* Drosophila *Genetics and the Experimental Life*, Chicago & London, University of Chicago Press

establish whether they are necessary or not. Recalling the Occam's razor, maybe it is no use and sometimes misguiding to multiply entities when there is no need. We will first remind what is understood by the term model organism, and justify why *Xenopus tropicalis* is a very interesting model organism. We will analyze the term chassis in order to emphasize the epistemological differences that can be made between a chassis and a model organism, and therefore wonder if *Xenopus* matches those characteristics.

R. Burian[5] suggested four characteristics defining what a good model organism is: a) the organism should be useful to realize a certain objective of research, b) easy to manipulate regarding the topic of the research, c) there should exist a large amount of experimental resources coming with the organism such as genomic data, and d) the organism should represent a class of organism with a specific interest. Though the practical usefulness of a model organism is mainly the large amount of data gathered around it, it is important for biology to have many different model organisms, representing different nods in the phylogenetic tree and remembering us that contrary to what François Jacob once said, what is true for *Escherichia coli* isn't necessary true for the elephant. As seen with the foreword, frogs have been by the past a quite useful organism for biology, for reasons different from those invoked before and on which we will develop in another part of our reflection. The old martyr of experimental physiology[6] has been progressively replaced by the now favorites model organisms of molecular biology and experimental medicine, which are the fly *Drosophila melanogaster*, the bacteria *Escherichia coli*, the plant *Arabidopsis thaliana* and the mouse *Mus musculus* (these are the most popular).

However batrachians in the name of *Xenopus tropicalis* are still widely use and might come back in the limelight. In 2010 *Xenopus tropicalis*' genome has been fully sequenced[7] and the frog enters in the realm of postgenomic model organisms. The frog slowly replaces its counterpart *Xenopus laevis* presenting interesting features for various kinds of research. *Xenopus laevis* was widely used as a model organism in developmental biology, cell biology, toxicology and neurology since the 1950s, being a particularly attractive model because of its manipulability and the size of its embryo, visible by naked eye. Without being a mammal, this vertebrate is evolutionary close enough with human to give us expandable results[8]. However, *Xenopus laevis* is a tetraploid and has a quite slow rate of reproduction, reaching its sexual maturity around one or two years, whereas *Xenopus tropicalis* is a diploid reproducing twice as fast[9] which makes it a more practical model, especially for genetics and for an iGEM

[5] Quoted by Rheinberger, 2006 Réflexions sur les organismes modèles dans la recherche biologique au XXe siècle, in Gachelin G., *Les organismes modèles dans la recherche médicale*, Paris PUF, p47

[6] Holmes F.L., 1993 The old martyr of science : the frog in experimental physiology, *Journal of the history of biology*, 26: 311-328

[7] Hellsten, U., Harland, R.M., Gilchrist, M.J., Hendrix, D., Jurka, J., Kapitonov, V., Ovcharenko, I., Putnam, N.H., Shu, S., Taher, L., Blitz, I.L., Blumberg, B., Dichmann, D.S., Dubchak, I., Amaya, E., Detter, J.C., Fletcher, R., Gerhard, D.S., Goodstein, D., Graves, T., Grigoriev, I.V., Grimwood, J., Kawashima, T., Lindquist, E., Lucas, S.M., Mead, P.E., Mitros, T., Ogino, H., Ohta, Y., Poliakov, A.V., Pollet N., Robert, J., Salamov, A., Sater, A.K., Schmutz, J., Terry, A., Vize, P.D., Warren, W.C., Wells, D., Wills, A., Wilson, R.K., Zimmerman, L.B., Zorn, A.M., Grainger, R., Grammer, T., Khokha, M.K., Richardson, P.M., Rokhsar, D.S. 2010 The genome of the Western clawed frog *Xenopus tropicalis*, *Science*. 328:633-636

[8] And it is the phenotypic resemblance that historically brought frogs into the hands of experimental biologists like Swammerdam

[9] Bringing Genetics To Xenopus: Half The Genome, Twice As Fast University of Virginia. Retrieved 2009-10-24: http://faculty.virginia.edu/xtropicalis/overview/intro.html

project. Though the size of the egg is a bit smaller (< 1mm), it still is visible by naked eye and quite practical to microinject.

Xenopus tropicalis *as a chassis?*

Thus *Xenopus tropicalis* clearly fits the characteristics defining a good model organism. A large amount of data exists on it as its genome has been sequenced, a large variety of studies are already done on *Xenopus tropicalis* and it is known to be easy to manipulate (characteristics b), c) and d)). If we keep an eye on Burian's criteria, being a chassis is a specific mode of the first one: being useful to realize a specific objective of research. As a chassis is a specific kind of a model organism, directed toward a specific use, we have to investigate more closely what it is expected from a chassis to get the interest of bringing *Xenopus* in the realm of engineering. *Xenopus* is not only interesting for fundamental research, indeed it is an important vertebrate for medical and environmental purposes, like gene therapy[10], drug discovery and environmental risk assessment, like the Watchfrog company.

Synthetic biology introduced the term "chassis" as a new way to refer to an organism. The metaphor is quite suggestive, in French the term appeared in the end of XVIIth century in joinery and is today more specifically known when talking about cars: the chassis is the rigid structure on which the various elements constituting the vehicle are fixed. This definition of a chassis can be kept and slightly developed when talking about living things: in synthetic biology, the term chassis primarily concerned bacteria, and specifically *Escherichia coli*. A chassis is meant to receive designed devices composed of standardized parts realizing specific functions. Thus a chassis is meant to become a living tool, such as a biosensor (giving us reliable results concerning the state of an environment), a biological factory (producing materials of values, like drugs or fuel) and a cleaning machine doing bioremediation. Even when used for more fundamental purposes (exchanging information, counting), the interest of chassis is to be the container of any application that we can imagine being possibly implemented in it. As the parts implemented in the chassis aimed to be standardized and as the chassis is aimed to be inserted in an industrial system of production, the chassis as to fit some criteria of technological artifacts, as safety, efficiency, reliability and profitability[11]. These are, according to M. Bunge, the epistemological principles driving technology.

These principles of technology applied to living beings make some of us feel uncomfortable, especially when it starts dealing with animals. Not that we are absolutely against animal experimentation, but we are not sure that chassis is a term epistemologically relevant concerning animal biotechnology. After all modifying genomes in order to change the properties of a living being did exist before synthetic biology. Rationally designing animals is not new; mice have been deeply modified in order to serve science since the "construction" of OncoMouse[tm] in the mid-80s and these operations lead to intricate controversies on patent laws[12]. Engineering a synthetic, orthogonal hormone as a communication device certainly

[10] Ymlahi-Ouazzani, Q., Bronchain, O.J., Paillard, E., Ballagny, C., Chesneau, A., Jadaud, A., Mazabraud, A. and Pollet, N. 2010 Reduced levels of survival motor neuron protein leads to aberrant motoneuron growth in a *Xenopus* model of muscular atrophy. *Neurogenetics* 11:27-40 doi : 10.1007/s10048-009-0200-6

[11] Bunge M., 1966 Technology as applied science, *Technology and culture*, Vol 7, n°3, summer

[12] The Harvard Oncomouse is thus widely seen as a "case" of bioethics which lead different decisions depending on the jurisdiction. http://www.wipo.int/wipo_magazine/en/2006/03/article_0006.html

provides an interesting tool to do research on *Xenopus* and maybe helps reducing the number of tadpoles needed to test drugs and cosmetics in biotechnological companies. But we have to make it clear that *Xenopus* is not a tool, it is an animal, an organism on which we are doing test.

This statement implies some epistemological differences, a tool or an instrument is well known artifact that gives the out-put corresponding with the in-put. We are not interested on what is happening in it. Chassis gives us the idea of a framework which had no other meaning or end but supporting man-made objects. A chassis is nothing by itself, has no end in itself, it is something that has to be built on. It is not interesting as we already know it because we built it in a particular purpose. There is no adaptation, no research to do on an already existing chassis as everything is already design to be functional. Hence we believe that this term might be misguiding. The chassis is an ideal of something like the minimal cell with the minimal genome designed to sustain living properties. By forcing the idea, one might include *E. coli*, *B. subtilis* or some other very well-known bacteria. But it seems quite inappropriate for more complex forms of life which development and organization are so far from our understanding. For this epistemological reason (and others we will develop latter) we prefer keeping the term model organism against the chassis idea. In our sense it provides a better idea to our work and don't deny the complexity of life properties. A model organism is an organism we are trying to know, it is a being that helps us increasing our knowledge about nature and about us[13]. The term chassis creates some epistemological and ethical confusion, what is the tool, synthetic biology's products or the beings modified by synthetic biology?

We discover with great interest on the eve of the wiki freeze the philosophical work of Pablo Rodrigo Grassi and the FreiGEM 2012 team. They did a quite interesting and accurate epistemological analysis of the concept of "living machine" as a core concept of synthetic biology. We deliver here the personal conclusion of P.R. Grassi:

"While I am indeed sympathetic to the practical work of synthetic biologists, I need to object to its underlying epistemology. One can still heal diseases, produce biofuel, build biosensors and so forth, without using analogical expressions like 'living machines', saying that we only understand life when we construct it and arbitrarily determine what belongs to life and what does not. The notion of life as a machine is not self-evident and it would be negligent to persist in it without further revision."[14]

We believe that the chassis metaphor is quite concern by this conclusion. We invite the reader interesting in an analytical approach of the "living machine" metaphor from the point of view of philosophy of language, biology and technology to have a closer look to FreiGEM report.

In last resort, one could say that chassis is a specific term of synthetic biology, a reference for the community principally used like a brand or a slogan. Nothing to make fuss about, and scientist are well aware of what is a tool, what is an organism and so on. However, when this is put in regard with reflections of the non-innocence of metaphors, we may prefer keeping the old terms such as model organism, which do not completely blot living beings out of our representations.

[13] Weber M. *Philosophy of experimental biology*, p171

[14] Grassi P.R. 2012, "Living Machines", Metaphors and Functional Explanations, p26 version online: http://2012.igem.org/Team:Freiburg/HumanPractices/Philo

But before arguing against the use of the term chassis especially when referring to animals, we have to spend a few times on the theories of animal ethics and the various aims of animal biotechnologies.

Free the frogs!

Why should ethics concern animals?

We will present two ways of integrating animals in our moral community with the different consequences drawn for animal experimentation. The two authors, Tom Regan and Peter Singer are generally considered as classics or foundations of animal ethics. This overview owes much to two books, J.-B. Jeangène Vilmer's *Introduction à l'éthique animale* and the anthology *Philosophie animale* directed by H.-S. Afeissa and J.B. Jeangène Vilmer.

"The day may come when the rest of the animal creation may acquire those rights which never could have been with-holden from them but by the hand of tyranny. The French have already discovered that the blackness of the skin is no reason why a human being should be abandoned without redress to the caprice of a tormentor. It may one day come to be recognized that the number of the legs, the villosity of the skin, or the termination of the *os sacrum* are reasons equally insufficient for abandoning a sensitive being to the same fate. What else is it that should trace the insuperable line? Is it the faculty of reason, or perhaps the faculty of discourse? But a full-grown horse or dog is beyond comparison a more rational as well as a more conversable animal, than an infant of a day or a week or even a month, old. But they were otherwise, what could it avail? The question is not, Can they *reason*? Nor Can they *talk*? But, Can they *suffer*?"[15]

Before entering into the various theories we wanted to quote this famous reflection of J. Bentham, an utilitarian philosopher of the XVIIIth century. Bentham theory of moral is widely recognized as one the first hesitation to rationally integrate animals in our theory of justice[16]. This quote introduces the basics references on the debate: the parallel made with racism and slavery, the disqualification of reason and languages as pertinent criteria to determine the limit of the moral realm, the temptation to define justice as the equality of human and non-human animals, equality based on the following criterion: the ability to feel pain. The parallel made with racism and slavery will be drawn again in 1970 with a semantic consequence, the creation of a neologism: *speciesism*. The analogy is drawn with racism and sexism. The term has been introduced by Ryder in 1970 in Oxford[17] and exposes the discrimination between being on the arbitrary criteria of their species. A quite simple case can illustrate this concept: many people would prefer that animal experimentation is done on rats rather than on cats on the simple basis that rats are disgusting and cats are cute.

Tom Regan is the most radical of the three authors we will present here. His approach is part of a deontological way of thinking ethics. This involves a clear definition of what is good and what is bad based with which you can't negotiate. A basic statement reflecting a deontological approach would the commandment "You shall not murder". Tom Regan considers that each subject-of-a-life has an intrinsic value. For this reason it is morally wrong to consider animals "as supplies at our disposal, to be eaten, to be subjected to surgical experiments or to be exploited or money or sports"[18]. The only way according to Tom Regan for the animal

[15] Bentham J. 1781, *An Introduction to the principles of morals and legislation*, Chapter 17

[16] J.-B. Jeangène Vilmer, 2008, *Introduction à l'éthique animale*, Paris, Presse Universitaire de France, p 33

[17] *Ibid.*p 45

[18] Regan T. Pour les droits des animaux, in H.-S. Afeissa & J.B. Jeangène Vilmer (dir), *Philosophie animale*, Paris, Vrin p162

condition to evolve towards a more respectable treatment is to give them a special place in the law. Hence, T. Regan defends the concept of animal rights, a quite radical concept, the only one that could help establishing a coherent and rational justice with regards to the foundation of justice. Indeed, according to the author, a theory of justice based on the capacity of reasoning and talking would exclude from its realm children and some of the most handicapped people. Concerning our subjects, animal experimentation and animal biotechnologies, T. Regan would defend an abolitionist position that can't be debated in reason of his deontological perspective.

Though the title of Peter Singer's book, *Animal Liberation*, may seem quite aggressive it is far more moderate than Regan's theory. Peter Singer develops a utilitarian theory in the continuation of Bentham. P. Singer doesn't want to mix justice with morality, talking about "animal rights" doesn't mean much to him as rights have to be link with duties and responsibilities: animals can't fulfill them. Neither can children nor strongly handicapped people, this is why they have tutors, people responsible for them. Nevertheless, P. Singer still defends a form of equality between human and non-human animals, which is called an equality of interest. This theory is based on the ability for animals to feel pain, to the interest of avoiding pain and seeking pleasure. Hence we have to do our best to respect those interests. This means reducing animal experimentation, breeding cows and pigs in good conditions, forbidding bullfights and other cruel games involving animals. But equality of interest doesn't mean equality of life value or equality of treatment. Some species have more interests than others, for example they need forms of society, communication etc. As the principle is too privilege the maximum of interests, might seem to be better treated than simpler. This theory implies to rethink our relation to food production, drug and cosmetic test on animals for developing a less excessive system respecting as much as we can the interests of the beings we are living with. As this theory is utilitarian, what we can and cannot do can be, to some extent, negotiated. We have to "calculate" the interests involved in our actions and established if there is a gain or not.

These two approaches of animal ethics, despite their differences, have in common to emphasize on the political aspect of the animal question. Taking seriously animals as beings that deserve a moral care implies changing many of our habits of consumption. These required changes are quite close in their quality to those recommended by many ecological theories: being less excessive and more attentive the world in which we are living. All this get quite interesting if we rapidly sum-up the ambition of animal ethics and ecology: making life better, one being at a time. Doesn't it sound familiar?

Animal biotechnology and human/non-human relationships.

Melvin Kranzberg, professor of history of technology and founder of the Society for the History of Technology, reached posterity through the formulation of six laws concerning technology which he called "The Kranzberg's laws"[19]. We will use the first law as a starting point on the relation of animal biotechnology and ethics. The first law states: "technology is neither good nor bad; nor it is neutral"[20]. This statement is crucial to be well understood by engineers and bioengineers, one can't state that technology is neutral, that everything relies on

[19] Kranzberg M. 1986, Technology and history: "Kranzberg's laws", *Technology and culture*, 27-3, p544-560
[20] *Ibid.*p545

the shoulder of the people using it. Once we are bringing new artifacts or entities into the world we can't declare that we don't have any responsibility of what will be done thanks to them or because of them. Bringing new entities to world open possibilities and creates norm that didn't exist before. Technology is generally presented as a solution to a problem and most of biotechnologies could be said to be produced in the same state of mind, making the world better. This will be, at least, the way they will be presented to the public. This aim of technology has many impacts on our ways of being. It changes our relation to time, space and society (through clocks, trains and social-networks), it gives us the possibility to be related to any part of the world and organizing international contests with real-time communication through internet.

However synthetic biology is still building itself on strongly anthropocentric values. These values are stated in the first fundamental canon of the American Society of Civil Engineers' Code of Ethics : "Engineers shall hold paramount the safety, health and welfare of the public and shall strive to comply with the principles of sustainable development in the performance of their professional duties." The attention to the principles of sustainable development was added in 2009, as explained in the footnote of the document. This novelty is quite important as it highlights the need of an evolution of our relation to non-human beings. The environmental crisis and maybe pressure from society compels technology to care for a "sustainable development". Synthetic biology appears to have in mind a quite similar understanding of ethics. Ethics is widely link with safety and security, with the issues of bioterrorism and the avoidance of environmental leaks. The formulation of the human pracice on the iGEM site also encourages this conception: "Will the world be a safe place if we make biology easy to engineer?. Those questions of environmental safety and risk assessment are quite difficult to deal with, and we are glad to see that the project of Paris Bettencourt was to take them seriously through a study of horizontal gene transfer. It seems to be too often taken as granted with some killer switch assumption.

But imporoving human welfare from an anthropocentric perspective can have for consequences a deep blindness of the environment in which we are embedded. The history of animal ethics testifies of the limits of a blind fulfillment of human diseres. The animal issue was triggered in UK by Ruth Harrison's book, *Animal Machines: The New Factory Farming Industry* published in 1964. From this book rises a campaign against industrial farming giving birth in the early 70s to the concept of "*speciesism*", and slogans around "animal liberation" "animal rights". The main works structuring the animal movement were published between 1975 and 1983; Peter Singer's *Animal Liberation* and Tom Regan's *Animal Rights and Human Obligations* (1976). *The Case for animal rights* (1983). The renewal of the attention of animal, though imposed to the practice by laws and ethical commitees, is not necessary integrated in the scientific reflection. A closer look at stories of "new" animals brought into living reveals that our responsibility concerning those beings isn't well established at all. Raphael Larrère tells the story of two hybrids, Agrostis, a transgenic crop, and Lucifer, a transgenic and cloned bull. The story of Lucifer tells something that we need to be well aware of as we decide to make living being and specifically animals easier to engineer. Lucifer was the only survivor of numerous embryos cloned and genetically modified, the fruit of a long and tough labor. However, when grew old, the bull was getting really aggressive and dangerous. It was getting really complicated to take care of it. After long debates, INRA's scientist decided to euthanize Lucifer. This case is interesting as it questions the responsibility of the creator on the creature. Don't we have duty towards being we brought to the world? Shouldn't we try to anticipate such situations? It also reminds that the questions raised by synthetic biology are not new. As technologies/biotechnologies shape the world we live in,

shape the society we live in, they also shape ethics. Technologies progressively address new ethical needs, as can be seen in the fifty last years. Animal ethics is an instance among many others, nearly insignificant comparing to all that has been thought after the second world concerning nuclear or the development of bioethics since the early nineties (Bioethics has for object what can or can't be done to human. It is not turned towards animals.)

Some of the members of the team think that synthetic biology gives us the possibility to renew our relation nature. By putting animals and bacteria at the center of our system of production we could make people more conscious of the implications of some excessive ways of living. To some respect, synthetic biology would be creating new symbiosis. Here comes the original formulation of this idea:

"To me, modifying frog or other organisms is not a way of controlling them, neither it is against the laws of nature (if it were, they would die anyway). To me, it is to develop a new symbiosis, which imply interdependency. In this setting, mankind will depend on these organisms, and will have to treat them well. People usually don't think of the dependence on animals synthetic biology creates, they only think about control (anthropocentric view). But I prefer being dependent on Bioengineered frogs, rather than on very polluting (and invisibly polluting and destructing) industries thousands miles from me. In a way, and counter-intuitively, synthetic biology would make us closer to nature and ecosystems..."

We said that technology has a lot in common with ethics. Technology and ethics concern our ways of living together, both of them seek to make human condition better, they are both shaping one another. There is a real good intention through the iGEM contest, and we don't mean to disqualify this intention. We just wish to underline that intentions can lead to no good if it's *a priori* are not questioned, if the façade speech and the backdoor activity lack of coherence. Intentions and technologies aren't enough to make the world better, they have to be accompanied by an extension of our moral judgment, of our capacity to be attentive to the beings living with us. Human beings need to understand that they are not the center of the world nor the top of natural evolution. Could synthetic biology play a role in the change of habits and consciousness?

Through its "making life better"/"saving the world" rhetoric, synthetic biology acknowledges the existence of important difficulties of our models of development. What some call environment and economic crises. Biotechnologies may bring some interesting tools and dynamics helping us to deal with those crises. However technology is not salvation, one does not bring another world without thinking through its relation to it. If synthetic biology aims at being part of the establishment of another world in which new entities will have an active role, it has to start rethinking our relationship to living beings in other ways than means to achieve an end. Various stories may show that the mere mean to an end relationship is too blind to be satisfying. As technology is not value neutral, we have to be quite conscious of the values we want to bear if we don't to enter in destructive marketing and communication propaganda which brings defiance from society toward biotechnologies (especially concerning food, drugs and energy).

Technology and ethics are normative, technology and ethics concern our ways of living together (see all the iGEM's project about communication etc.). There is a real good intention

through the iGEM contest, and we don't mean to disqualify this intention. We just wish to underline that intentions can lead to no good if it's *a priori* are not questioned, if the façade speech and the backdoor activity lack of coherence. Intentions and technologies aren't enough to make the world better, they have to be accompanied by an extension of our moral judgment, of our capacity to be attentive to the beings living with us. Human beings need to understand that they are not the center of the world nor the top of natural evolution. T. Regan certainly makes a point when he reminds us that we do not have a right over nature.

A chassis, really?

If one accepts what has been developed in our two firsts parties, we believe that he must already be convinced that chassis isn't a good metaphor for referring to non/human animal such as *Xenopus tropicalis*. However it is important to insist on this part of our development we expect to do two separate things. Insist that the chassis metaphor isn't an adequate term to refer to animals (and certainly living beings, but this goes beyond our project) from both epistemological and ethical point of view; and present an original work recalling the service done by frogs to science, as a special acknowledgment of our collaborator *Xenopus*.

Metaphors are not innocents

We have to take keep in mind that there is a performative dimension of language, the words we use influence the ways we act, they tend to open some dimensions of our practice and close others. Cows, pigs and chickens do not require the same treatment than meat factories. It doesn't deploy the same universe, the same images. And publicity understood the importance of the representation we have concerning what eat and do not eat. The awareness campaign campaigns showing animals mistreated emphasize on what is behind the cow you eat: the construction and demolition of meat factories. We can find many example through history in which reality is transformed by language in order to allow practices that couldn't be done before. Richard Lewontin, insists on the limit of some metaphors that limit the possibilities opened to research.

In *The Triple Helix* (1998) the geneticist of development Richard Lewontin explains how different metaphors describing the development (word which is already a metaphor) of an organism influenced the ways we understood embryogenesis and the interaction between the organism and its environment across the XXth century bringing molecular biology to the dream that "decoding" the genome will be the key giving us the full understanding of life and a way to cure most of the diseases.

"While we cannot dispense with metaphors in thinking about nature, there is a great risk of confusing the metaphor with the thing of real interest. We cease to see the world *as if* it were like a machine and take it to *be* a machine. The result is that the properties we ascribe to our object of interest and the question we ask about it reinforce the original metaphorical image and we miss the aspects of the system that do not fit the metaphorical approximation. The price of metaphor is an eternal vigilance"[21].

During the debates many biologists suggested that metaphors were necessary to take some distance with the animal studied (or in case of slaughterhouse, the animals to cut up), however one might that this necessity doesn't reveal the issues of habits and the loss of attention to others that it may create.

[21] *Ibid.* p4

Historical praise to the frog as a martyr of science

The French historian of science Christine Blondel and physicist Bertrand Wolff wrote a historical praise to the frog on a popularization website devoted to the Ampere and the discovery of electricity[22].

This praise represent a kind of special attention that can developped concerning any model organism. Acknowledging the history of being is a way among others to recall our dependance on its existence, dependance requiring a special attention to its interests.

We present here a traduction of their work :

"The frog is kind-hearted…

Without it, would William Harvey discovered the blood flow? The cold-blooded animals like toads or frogs have a slow heart that is easier to analyze than mammals. It is by looking at them that Harvey made his first observations. In his work Motus Cordis, he assures that the blood is expulsed by the heart to the artery and goes by through the veins. "This is how I start to wonder if there was a circulatory move of the blood". But, Harvey died before finding out what becomes to the blood between the arteries and the veins.

The lung devoted to Science…

Once again, we have to thank the frog for solving the enigma of the blood circulation. Observing under a microscope le lung of a frog, Marcello Malpighi noticed very thin blood vessels - the capillaries - that link the small arteries to the small veins.

In 1661, he wrote :

"Things are much easier to see with the frogs (…). The microscopic observation revealed things even more prodigious (…). I clearly saw that the blood got divided and circulated in tortuous vessels"

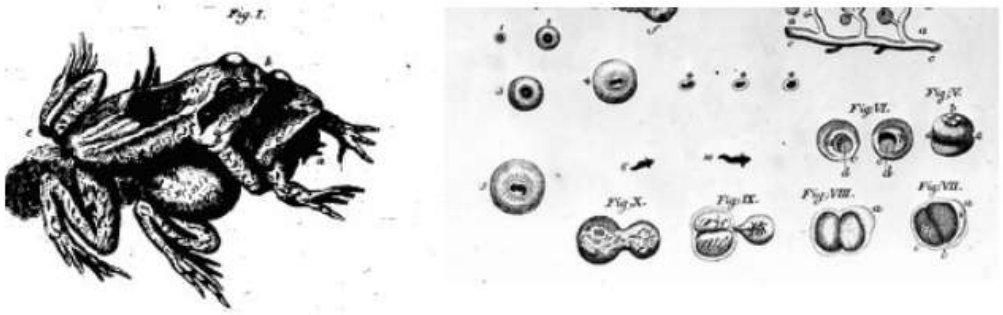
It is by crossing the lungs that the veins' blood goes back to the arteries. But, to get this result, Malpighi admitted, even glorified himself:

"I have almost scarified the entire race of frogs, something that never happened before, even during the furious battle between rats and frogs described by Homer".

… And sexual practices submit to scientists' voyeurism.

Theories of "generation" – that is of reproduction – had aroused passionate quarrels. One of them was about fecundation: what part plays the man seed? The frog, as a very common animal, and reproducing quickly, has the merit of transparence… It reproduce by external fecundation, the eggs laid by the female are straight after recover by the mal seed.

[22] Version française en ligne :
http://www.ampere.cnrs.fr/parcourspedagogique/zoom/galvanivolta/eloge/index.php



Jan Swammerdam, *Biblia naturae*, Leyde, 1737-1738 [le manuscrit date de 1679]

In the XVII th century already, Jan Swammerdam was interested by the frog's reproduction. With a small magnifying lens, he observed the cellular division of a fecundated frog's egg. In 1768, Lazzaro Spallanzani dressed a male frog with a little panty made of leather, not without difficulty, because the animal tried to get rid of it!

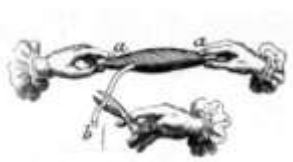
"What assure the whole, is that I've putted some straps to these panties. I slipped it on the harms of the male frog, under his head, between his body and the female's one"

The females mated with these males wearing panties freed their eggs, but these ones decayed and didn't transform in tadpole. But, inside the panties, Spallanzani found drops of transparent liquor. He took a sample of virgin eggs in a frog's ovary, knowing by experience that they can spontaneously develop. He damped them with the collected seed and noticed a few days after that the eggs developed as well as if they were naturally fecundated by the male.

"He just came to realize the first artificial insemination in a laboratory" wrote Jean Rostand. This series of experiences – with more than 200 frogs – allowed Spallanzani to ruin the hypothesis of fecundation at distance: the direct touch of the egg and the male seed is essential.

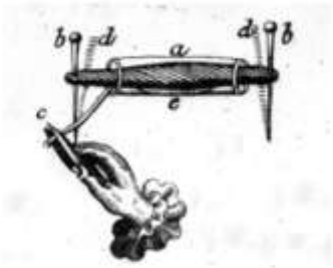
It has nerve, and tight!

In the middle of the XVII th century, the Dutch naturalist Jan Swammerdam explored another field of life: the transmission of the nerve impulse. A frog can keep on swimming even if one's take away its heart, while it comes to a standstill if one's take away its brain. The blood circulation is not necessary to move (at least for the frog). In 1568 he demonstrated, in front of the Duke of Toscane, the contraction of the frog's muscle, separated from the frog with its nerve, under the action of a simple compression of the nerve. While there's no more links between the nerve and the spinal cord, the contraction can be repeated at will. Opposing to the most widespread hypothesis, Swammerdam concluded of his experience that the muscle contraction can't only be explained by the action of a fluid that flowed inside of the nerve, from the spinal cord to the muscle.



Jan Swammerdam, *Biblia naturae*, Leyde, 1737-1738 [le manuscrit date de 1679]

Une des expériences de Swammerdam sur un muscle de la cuisse de grenouille :
"si l'on irrite en b le nerf avec des ciseaux, ou tout autre instrument [le muscle se contracte] et rapproche les deux mains qui tiennent les tendons"



Une version plus précise de l'expérience : "mettre le muscle dans un tube de verre, a, et percer les tendons de deux fines aiguilles bb [...] dont on fixe la pointe dans un morceau de liège. Si on irrite alors le nerf, c, on voit le muscle rapprocher les aiguilles en dd ; quant au ventre du muscle, il devient considérablement plus épais [...] et bouche le tube [...]"

Box 1 : One of the experiences of Swammerdam on the muscle of a frog. "If one irritates the B nerve with scissors – or with any instrument [the muscle contracts] and move the two hands that handle the tendon closer"

Box 2 : In one more precise version of the experiment "put the muscle in a glass tube and pierce the tendons with two fine needle BB […] from which the tip is fixed in a piece of cork. If one's irritates the nerve C, one can see the muscle moving the needles closer in DD; while most of the muscle becomes considerably thicker […] and blocks the tube".

The details and conclusions of Swammerdam's experiments are not much diffused because, renouncing to science to focus to spirituality, he burned a part of his manuscripts. What's left will be publish half a century later, but his public demonstrations and his correspondence made his works known by European scientists. The extreme sensitivity of the frog and the facility by which one can isolate its nerves had made it a preferred subject in the study of the nervous command. In the second part of the XVIII th century, the nerves of the frog's tights are excited by pressure, by "irritation", by chemical action of opium or curare, or more, by the direct use of electricity.

It is "the most delicate electrometer discovered until now" (Galvani, 1786)

The new stimulant discovered by Galvani – a distant spark – caused a general astonishment and new wave of researches. We won't tell here the story of the adventures that took Galvani's frogs to the Volta battery. But, we should note that Volta joined Galvani in his praise to the precious batrachians:

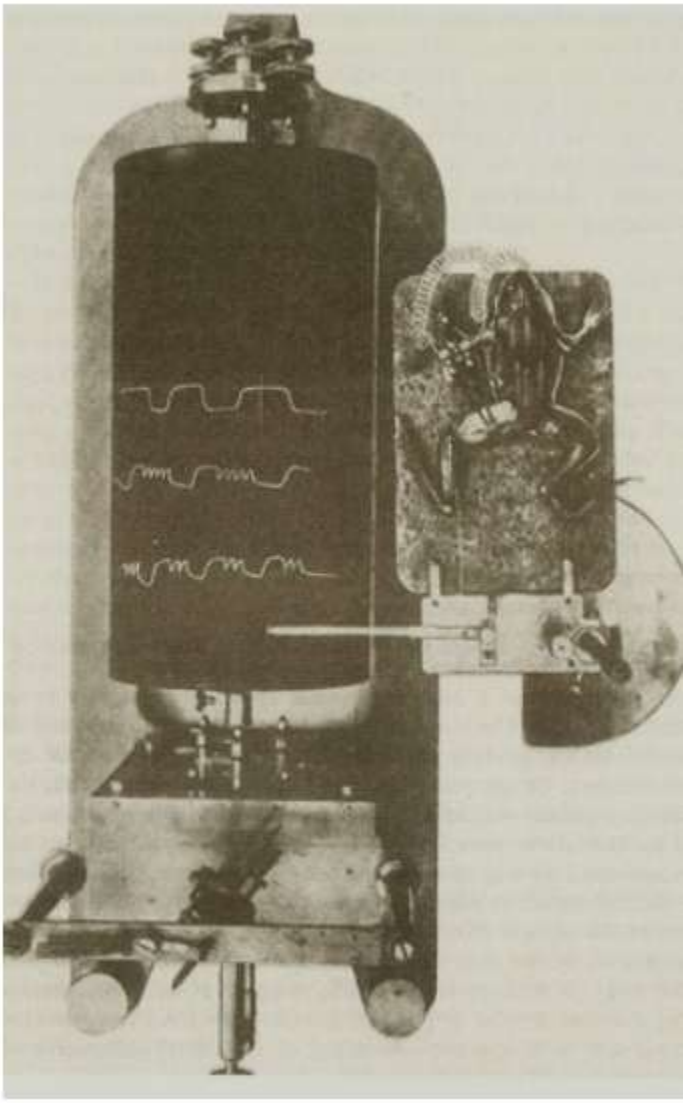
"I've chosen the frog among any other animals, because it is gifted by a very sustainable vitality and because it is very easy to prepare it". (Letter to Cavallo, 1793)

The "galvanists" in favor of animal electricity and the "voltaists" in favor of the metallic electricity enrolled the animal in their controversy. The great traveler and naturalist Alexander von Humboldt transported in his luggage, "even riding a horse", pliers, scalpels, and metallic patch to defend the thesis of animal electricity on local frog, always accessible in neighboring pond.

Today, animal manipulation is no more allowed in schools, and public demonstration had to be replaced by another media.

Pioneer of the wireless communication, it catches electromagnetic waves!

The frog's career in electricity didn't end in 1800. After the announcement of the existence of electromagnetic waves by Heinrich Hertz at the end of the 1880's, many scientists and inventors looked for diverse detector of these mysterious waves. Thus, in 1912, Lefeuvre, professor in physiology at the University of Rennes, realized a "physiological detector", capable to detect waves of telegraphic wireless emitted in Paris. Before being able to diffuse speech and music, the Hertzian waves transmitted Morse code telegraphic signals. The core of Lefeuvre's apparatus is nothing else than a frog, which tight contracts at the signal reception! One dash: an extended contraction, on dot: a brief contraction.



← Le "détecteur physiologique" du Professeur Lefeuvre.

Sur cet enregistrement, la grenouille a détecté trois fois de suite un "trait" suivi de 4 "points", puis un "point" isolé. ↓

In its bowl, it predicts weather

The popular wisdom in weather prevision uses many of sayings that refer to the frog:

“If the weather is nice, the frog croaks on the banks of its ponds”; in the vase, it digs, if the bad weather is coming”.
“Frogs that croak at day, rain in the three days”
“When frogs sing at night, in the morning, the sun shines”
In the 70’s, on the French radio Europe 1, the famous hoarse-voiced meteorologist Albert Simon awarded the quality of his prevision to his frog which, in its bowl, went down or up its ladder in accordance with the variation of the barometer.

A “privileged fragment of the living world”

Let’s go back to life science. The study of the parthenogenesis - that is the reproduction without male - by the biologist and popularizer Jean Rostand was also done with frogs. By inflicted an appropriate trauma on a non-fecundated egg, one can obtain the cellular division of this egg. Thousands of toads, frogs and tadpoles were submitted to these experiments:
“We can study all the problems on frog. People think that frog is a small subject but it isn’t. All biology is in a frog. We can study cells, sperm, eggs, parthenogenesis, etc. Lastly, even the mutation and the heredity. We can study everything on a frog”, it constitutes a “privileged fragment of the living world”.
A new form of reproduction without male, the cloning, was realized for the first time in 1962 on a frog, before the ewe Dolly, by the introduction of the nucleus of an intestinal cell in a nucleus-free egg.

We can let George Canghuilem, philosopher and historian of life science, conclude that:
“The frog was, so to speak, a good girl for physiologist; they used it a lot and it was very useful to them”. We can add that if the frog finds a good place here, it’s because it was also a good girl for “electricians”.”

Should we meet again Xenopus tropicalis in iGEM?

Our team introduced *Xenopus tropicalis* as a new model organism in the iGEM contest. We believe it is an interesting collaborator for synthetic biology, as it as quite well known and already used in biotechnologies. Working with *Xenopus tropicalis* larvae constitutes a progress in animal experimentation as long as we take care not to let the larvae turns into its adult form. Without being developed enough to feel pain (as far we know until now), the larvae is an interesting alternative to the use of more sensible beings. However some of us believe that animals (were they larvae or grown up) shouldn't be involved into animal experimentation dedicated to our comfort (for examples, cosmetics). Furthermore the animal should be recognized as a being and not reduced as a tool. This means keeping appropriate languages and practices and thus maybe avoiding the dangers of the metaphor. Maybe the language thing still seems quite a neutral for some of you. However keeping a simple vocabulary doesn't seem an objective too demanding.

As we said in intro, this article doesn’t reflect the opinions of the whole team, however those questions were debated and no consensus were fully accepted. His tone might be a bit provocative and in rupture with the enthusiasm characteristic of iGEM projects. However The dividing topic of the term "chassis" was represented by our T-Shirts: some of us believed that the term chassis should be maintain, as a mark of the identity of synthetic biology and it’s engineering methodology. Others believe that it should not be kept, for the various reasons said through this investigation: the term chassis does not seem epistemologically or ethically relevant. The evolution of technology may be going too fast regarding the human capacity to use it wisely. We think we live in excessive societies, blindly consuming natural goods and hardly mastering all the technology existing around us. In those conditions, the race to innovation and novelty is likely to bring new excesses creating new problems. Developing business dealing with the consequences of our excesses can’t be a sustainable solution... This human practice had for ambition to challenge the usual conception of beings in synthetic biology through the case of the frog *Xenopus tropicalis*. It was an occasion to deal with theories that rarely enters in the laboratory because they are mostly upheld by opponents to synthetic biology or revolutionary technoscience (the development of synthetic biology is quite similar in communication as the one of nanotechnologies, except for one remarkable thing: synthetic biology opened its doors to embedded humanities).

Should we meet again *Xenopus tropicalis* in the iGEM contest? The question is open, most of the team believe that it should be a good thing for synthetic biology to develop better tools for researches on vertebrates, two of us are more baffled... iGEM seems too much about "living machines", chassis, tools and living factories, to be a welcoming place to non-human animals as beings to be attentive to... but who knows, we might be wrong?

Acknowledgments

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And a special thanks to *Xenopus tropicalis* whom was the starting point of this adventure and thanks to whom, in a particular way, we all met each other.