Chapter 4

Biological Standards and Biosecurity: The Unexplored Link

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The issue of standardisation in Synthetic Biology has important implications at both the technical and governance levels. At the former, standardisation in biology (a still-ongoing process) is expected to exponentially increase the potential of synthetic biology by democratising, easing and expanding our ability to engineer life. Indeed, it has to be stressed that Synthetic Biology is -or at least aims at being- a fully engineering discipline. And engineering, from industrial to electronics, largely relies on standards. A standard is a part, piece, device or procedure with well-established properties, and which can reliably be used in a broad range of industrial applications. Standards are often considered as universal components, in such a way that their constant properties allow a world-wide use. A well-known example of standard parts are nuts and bolts. Indeed, the onset of the industrial revolution was associated with a bloom of different designs of nuts and bolts, with different sizes and thread pitch. It soon became obvious that a standardisation of nuts and bolts was required: standard nuts and bolts were born.

Today, standards are everywhere in our technological civilization. From the screws of our cars to the lids of plastic water bottles, the industrial world we live in would simply be impossible without the use of robust, reliable and standard components. In Synthetic Biology, however, the standardisation process is still in its infancy. Despite the enormous efforts carried out so far to develop and use standard biological parts, plasmids or procedures, the challenge of standardising the biological realm has proved very difficult (Porcar et al. 2015). In this section, we will address the state-of-the-art of standardisation in biology, as well as the implications in biosafety and governance. In order to address this issue, it has first to be stressed than standardisation in the frame of synthetic biology can actually refer to two

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separate and different perspectives, that, for the sake of clarity, we name from now onwards standards *in* biology or standards *for* biology.

4.1 Standards in Biology

In contrast with the standardisation of the norms and regulations of the biosafety or biosecurity issues of synthetic biology (see standards for biology), standards *in* biology are those that are used *within* the discipline. These include, in a matryoshka-like hierarchy of complexity, the following levels amenable to standardisation: parts (i.e. short genetic sequences), devices (simple combinations of the former), genetic circuits, metabolic pathways, engineered cells and engineered cell consortia (or cell tissues). Beyond all those physical components, standards in biology can also refer to the protocols and techniques used in the discipline, as well as the human practices (manual movements, use of biosafety material, etc.) required to carry out any synbio experiment. Figure 4.1, extracted from the BIOROBOOST project, describes this comprehensive and hierarchical structure, which shows the complexity of the endeavor.

This comprehensive description includes all levels in SynBio that are amenable to standardisation. But the question is, do we really have standards at all those levels? The answer is negative, although it is true that major efforts have been made and are still ongoing in order to develop robust standards. In order to get a glimpse of the state-of-the-art in SynBio, two examples of standard parts can be considered: BioBricks™ and pSEVA plasmids.

Fig. 4.1 Standards in biology. From DNA part to the protocols and activities of the research staff, all levels are amenable to standardization. (Source: BIOROBOOST project website, <http://standardsinsynbio.eu>)

BioBricks are DNA sequences which conform to a restriction-enzyme assembly standard, used in SynBio (Smolke 2009). Biobricks were one of the former attempts to standardise engineering in biology, and they are certainly one of the most widely used components, with many scientific references reporting their use, advantages or deficiencies. The main reason behind this wide use is due to the international Genetically Engineered Machine (iGEM) competition (<http://www.igem.org>), in which hundreds of teams of mainly undergraduate students develop and present ingenious SynBio projects for which the use of BioBrick parts is imperative. Indeed, iGEM teams not only can use the thousands of BioBricks parts available in the Repository of Biological Parts (http://parts.igem.org/Main_Page) but are requested to provide at least one new part to the registry, in a potent and effective strategy to both broaden the number of available/registered parts and to foster the use of existing Biobricks. Unfortunately, the wide use of Biobricks has not resulted in broad acceptance outside the iGEM world. Moreover, a close analysis of the use of Biobrick-based standard parts by iGEM award-winning teams clearly shows that those teams tend to develop their own standards rather than using those already present in the Registry (Vilanova and Porcar 2014). This lack of trust in the reliability of BioBricks leads to the surprising result that re-inventing the wheel, rather than using standards, is the path to win a standard-centered competition (Fig. 4.2).

Another, very different, example of a widely used standard part in SynBio is that of Standard European Vector Architecture (SEVA) plasmids. SEVA consists of a set of shuttle plasmids developed by the team of Victor de Lorenzo (CNB, CSIC, Madrid, Spain). While most plasmids are only usable in a given bacterial species, shuttle plasmids in particular are functional in a relatively wide range of bacterial species. This means that those plasmids can be used, exchanged and transferred within and among many bacterial species. SEVA plasmids (pSEVA) have been made freely available to researchers worldwide. Since their publication, de Lorenzo's group has received more than 500 requests; more than 2000 plasmids have been shipped to 35 countries worldwide, and they have been used in research which yielded, for example, 277 (SEVA 1.0, Durante-Rodríguez et al. 2014) and 88 (SEVA 2.0, Martínez-García et al. 2015) citations (Esteban Martínez, personal communication). Interestingly, and in contrast with the case of Biobricks, pSEVA plasmids have thus found their way as standards in many SynBio's toolbox without a regulatory (i.e. iGEM's rules) requirement.

pSEVA plasmids could metaphorically be considered "double standards". First, their proven robustness and relatively wide use fit with the definition of standard; and second, the fact that they can be used in a broad range of bacterial species make these plasmids particularly "universal" (they can be interchangeably used in several bacteria). It has to be stressed, though, that the universality of bacterial hosts has both technical and biosafety/biosecurity implications. Regarding the former, this universality contributes to develop the potential of SynBio by facilitating genetic modification within different bacterial taxa. Regarding the latter, the question arises on whether this ease of modification of non-model bacteria (potentially including pathogenic ones) may contribute to an increased concern in terms of the biosecurity and biosafety of those and other technologies being enabled to trespass the "species barrier."

Fig. 4.2 Use of standards in a SynBio standards competition. The figure shows the number of parts from the Registry of Standard Biological parts used in the international Genetically Engineered Machine (iGEM) competition by 30 award-winning teams. An overwhelming number of parts are new ones/unverified and thus not standard. Original figure published in Nature Microbiology and available at <https://www.nature.com/articles/nbt.2899>

Fig. 4.3 Aspects of biological standardization as they relate to biosecurity

4.2 Implications of Standards in Biosecurity in Terms of Risks

The issue of biological standardization as it relates to biosecurity has not previously been addressed in detail. In the present chapter, we identify a series of aspects linked to standardization and their implications in biosecurity (Fig. 4.3).

4.3 Universality

An example of an almost-universal device is the smartphone. There are millions of them on Earth, and in many countries, most citizens have at least one. Smartphones are standard in the sense that, despite the existence of different models (or strains/species, in biology), they work in an equivalent way. Receiving or sending a Whatsapp message, for example, is largely independent of the smartphone used, because they all work alike with the app. Additionally, an informatic virus, a particular fake new or a geolocation involving smartphones could have an effect on all of them. In other words, the universality of a device is linked to the universality of the risk. Not unlike smartphones, making a standardized platform for SynBio would universalize the risk. If a given plasmid, virus or cellular chassis were made universally available, so would be the risk derived from malicious use.

4.4 Chassis and Trojan Horses

In Homer's Iliad, Greek soldiers entered the city of Troy hidden inside a wooden horse. It must be stressed that the horse was not the weapon, but the vehicle of the actual weapon (the army). Considerable effort was required to set in place the horse as a chassis of the weapon, but once in place, its further use became much easier (although there are no mentions in the Iliad of a further use of the horse). In the example above, smartphones were described as standard devices that may serve as chassis/Trojan horses. Biological chassis, provided that they are robust, easy to maintain and to amenable to modification, could also be considered as biological Trojan horses: inoffensive by themselves, but susceptible of being used as deliverers of bioterrorist actions because of their manipulability.

4.5 Breaking Down the Species Barrier

As we have stressed in the previous section, several currently ongoing efforts are successfully allowing microbial transformation by introducing plasmids in a range of different species (see the description of pSEVA above). The obvious implication in terms of biosecurity is that pathogenic DNA fragments could be inserted into harmless bacteria turning them pathogenic or, alternatively, pathogenic bacteria could be turned into more lethal agents by including certain biological circuits from taxonomically distant bacterial species.

4.6 Standards as Social Constructs

As a final remark, we strongly believe that it is important to be aware of a common misconception on the “inner” nature of standards. Robustness, reliability and ease of use are highly relevant features of any standard. That said, though, a standard must be acknowledged within a group of individuals on a basically arbitrary basis (see metric units, flag colours, and any other “conventional” standards). This has implications in terms of biosecurity assessment, since discussions tend to focus on the risk of biological parts *per se*, and not on the risk of standards *because they are standards*.

4.7 Final Remarks and Open Questions

As we have seen in this chapter, standardization in biology is a complex, still in process path that will be central for SynBio to fully develop its potential. Standardisation could finally make SynBio's promise come true and make biology easier to engineer. As we have described above, this fact will ineluctably be linked to an increased risk in the discipline because of the universality of the biological systems (and actors), their amenability as Trojan horses, and the possibility of easily breaking the species barrier. The question arising here is not thus whether advances in standardization will be linked to increased bioterrorism concerns, but to which extent the risk is proportional to the standardization level accomplished. This question does not only affect biosecurity but also biosafety. Consider, for example, the much needed biocontainment of potentially dangerous biological agents: are biocontainment strategies different in a standard-free vs. a standard-complete scenario?

As a general conclusion, the standardization of SynBio is a complex process, mostly still in its infancy. The success of this process will result in immense economic and societal benefits. However, the risks of SynBio in terms of biosecurity are only partially known, and the implications of the possible success of the ongoing standardization process in the biosecurity threads of this emerging discipline deserve further study.

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