PREFACE

This new book examines the latest research in the synthetic biology which refers to both: the design and fabrication of biological components and systems that do not already exist in the natural world the re-design and fabrication of existing biological systems. It also deals with Integrative biology is the study and research of biological systems. It does not simply involve one discipline, but integrates a wide variety of disciplines that work together to find answers to scientific questions.

As explained in Chapter 1, synthetic biology involves the design and construction of new biological components and systems, not already existing in nature, as well as the re-design of existing, natural biological molecules, structures and organisms for useful purposes.

Genetic and protein engineering tools, as fast and cheap DNA sequencing and synthesis, and protein evolution in vitro, would allow for rapid design, fabrication, and testing of systems.

"Chemical synthetic biology" is concerned with the synthesis of chemical structures such as proteins, nucleic acids, and other which do not exist in nature. An example deals with the so called "never born proteins" (NBPs), whose design and construction is aimed to understand why and how the protein structures existing in nature have been selected out, with the underlying question whether they have something very particular from the structural or thermodynamic point of view. Moreover, the NBPs may also have bio-technological importance, displaying, for example, novel catalytic and structural features not observed in natural proteins.

Synthetic biology could provide the tools and understanding needed both to develop and to expand "nano-biotechnology". Proteins can be rationally modified to bind to new, non-natural substrates, being adapted for nano-technological uses. There is no need to rely on the complexity of the immune system in order to conduct combinatorial searches for new peptides of this sort. The NBPs can be produced in laboratory by modern molecular biology techniques. The principle to produce NBPs is simple: if one makes a long string of DNA purely randomly, the probability of hitting an existing sequence in nature is lowest. Then this DNA is processed by standard recombinant DNA and in vivo expression techniques, thus obtaining a polypeptide that does not exist in nature, and when this polypeptide is globularly folded, a NBP is already obtained.

The phage display technique has been used to produce very large libraries of proteins having no homology with known proteins, and being selected for binding to specific targets. The peptides are expressed in the protein coats of bacteriophage, which provided both a

vector for the recognition sequences and a marker that signalled binding to the respective target.

Phage display can be considered as a practical realization of an artificial chemical evolution. As such, it is an useful tool for in vitro protein evolution, aimed to identifying new receptors and natural ligands for "orphan receptors", drug discovery (peptides might act as enzyme inhibitors and receptor agonists or antagonists, or otherwise modulate the enzyme/receptor's biological effect), "epitope discovery" (a new approach to disease diagnosis and vaccine development), design of DNA-binding proteins, source of new materials.

All this can be regarded as a kind of synthetic biology in that it involves the reshaping and redirecting of natural molecular systems, phage, typically using the tools of protein and genetic engineering.

Aquaporins (AQPs) play important roles in maintaining the water homeostasis. The anuran AQP family is known to consist of at least AQP0-AQP5, AQP7-AQP10, and two anuran-specific types, designated AQPa1 and AQPa2. In *Hyla japonica*, AQP2 (AQP-h2K) and two forms of AQPa2 (AQP-h2 and AQP-h3) reside in the tight epithelial cells of three major osmoregulatory organs: AQP-h2K in the kidney, AQP-h2 in the urinary bladder, and both AQP-h2 and AQP-h3 in the ventral pelvic skin. These AQPs are antidiuretic hormone-dependent AQPs. In Chapter 2, the authors describe in detail the anuran AQPs that have been identified to data and describe the molecular and cellular mechanisms underlying the terrestrial adaptation of anurans in terms of AQPs.

The ability to coordinate cellular responses in a population is critical to achieving certain behaviors in both natural and artificial systems. In bacteria, the coordination of cellular activity at the population level is implemented through quorum sensing. At their most basic, quorum-sensing systems are composed of three main components, making them amenable to genetic manipulation. In Chapter 3 the authors review the work researchers have done to isolate the components of these systems into well-characterized modules for use in the construction of genetic circuits that program complex cellular behaviors. Such modules have not only allowed for the incorporation of traditional quorum-sensing responses in host organisms, but have also allowed for the creation of a diverse array of novel responses based on cell-cell communication.

Synthetic morphology is a coupling of synthetic gene networks to output modules that change the shape or the morphogenetic behaviour of cells. In other words, it is the engineering of biological form. It differs from traditional surgical and tissue engineering approaches in that it is not restricted to forms that exist in nature and it works by making cells organize themselves into the desired form, rather than by manual manipulation of tissues. Synthetic biology has uses in basic science (eg testing hypotheses in developmental biology), industry (eg altering microbial associations and behaviours to improve the efficiency of fermentation, drug production and remediation of polluted soils) and medicine (engineering cell collectives for ex-corporo organ substitutes, engineering novel cell arrangements for repair of tissues or interfacing with artificial devices). Chapter 4 introduces morphogenetic 'primitives' that can be used, in different combinations and sequences, to produce engineered forms and that can be invoked by the expression of single molecules. Both bacterial and mammalian systems are described.

In Chapter 5, the study of the shell wall of Antarctic species *C. antarctica* Saidova, 1975 in SEM was carried on for the first time. The character and disposition of sand particles,

pseudopore openings and inner and outer organic linings and thin structure of the apertural collar was revealed. The character and position of the aperture of this species and of the genus *Conotrochammina* on which there were some contradictory data in the previous literature was clarified. The taxonomic position of the genus was changed: it was moved from the subclass Textulariana to the subclass Hormosinana according to the terminal position of its aperture beginning from the initial stage of its shell.

As discussed in Chapter 6, characteristics of feedback loops, i.e. closed interaction chains, can often be used as indicators of the kinds of dynamics a biological system can exhibit. However, published definitions of feedback vary widely, especially in their verbal descriptions of feedback. In particular, opinions differ as to whether the sign of links in a causal chain are given by the direction of the *processes themselves*, or *how process rates respond* to changes in system state. In the latter case, a self-effect (the direct effect of a state variable on itself) may appear qualitatively different depending on whether one considers the state-dependency of the *relative* or the *absolute* rate-of-change of the variable. A fourth definition uses "feedback" as a summary measure of all disjunct loops of action in the system.

For a system of ordinary differential equations, the authors' evaluation favors a definition of "the action of state variable x on y" as being positive if an increase in x causes an increase in the rate-of-change of y. Thus, the sign of an action is given by the corresponding element in the Jacobian matrix, evaluated at the current state of the system. No predictive power seems to be lost by abandoning the competing definitions of feedback, because all authors base their mathematical treatment on the Jacobian matrix.

Although many useful quantities can be derived from a dynamical model, not all of them should be called feedback. In the authors' opinion, a positive or negative term in the rate-of-change should be called just that. Similarly, per capita growth rates may often be adequately characterized as positively or negatively density-dependent. Lumping disjunct causal loops in a single feedback term seems misleading, because disjunct loops are not causally interconnected.

The state-dependence of process rates is a fundamental feature of natural dynamical systems, which makes this notion of feedback widely applicable. This suggests that verbal and conceptual explanations focussing on how process depends on state will be heuristically useful.

Advances in biotechnology have and are providing -for the analysis of events occurring at the molecular genome-wide level- innovative tools which change deeply the scientific and medical landscape of this century. These tools in fact are not only offering more detailed and abundant data, but, also a different -systemic- vision on how molecular mechanisms operate, with direct impact on our way to perceive the chemo-physical mechanisms that underlie drug discovery, therapy delivery, and ultimately medicine and health care. To effectively take advantage of the novel technologies, biology and medicine cannot be any longer two separate and distinct fields, they need to interact in order to bring the discoveries of the fundamental mechanisms of activity of the cells into applicative actions at the patients bedside. This is carried on by the so called *translational research*. Chapter 7 will present an introduction on the technologies that are promoting these profound changes, an overview on some of the most striking changes occurring in biology, pharmacology and medicine, the areas most closely related to health care. All these concepts will be used as the rationale to deepen and promote the use of the scientific approach to complementary and alternative medicine, an area too poorly explored and yet bearing the promise to offer alternatives in the authors' way to

approach diseases. This chapter aims at providing the background necessary to understand the scientific bases and current evidence that allow to perceive this area as a promising area of development, along with the limitations and issues that need to be solved.

Tissue engineering has emerged as a response to the problems associated with the substitution of tissues lost due to diseases or trauma. Nowadays, xenografts, allografts or autografts are commonly used to replace the damaged tissue. However, these options involve numerous drawbacks such as rejection, chronic inflammation and severe organ donor shortages. Tissue engineering keeps the promise to overcome these limitations by creating biological substitutes capable of replacing the injured tissue.

Cells, temporary 3D constructs and biochemical signals that trigger tissue regeneration cascades are the three major components for engineering tissues. The adequate combination of these components leads to the successful repair of damaged tissues. In addition to these three main elements, the use and development of bioreactors has come out as an essential tool for the study and achievement of engineered tissues under *in vitro* conditions while mimicking the *in vivo* environment.

Thus, tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences to develop biological substitutes that restore, maintain, or improve the tissue functions.

Chapter 8 discusses the challenge of creating engineered bone tissues by combining biological and engineering skills. In particular, it will review the issue of stem cell biology, the fabrication methods and artificial materials used for 3D constructs, the principal growth factors used in bone applications and the key role played by bioreactors. More importantly, it will highlight the interdependence of all these parameters and the need for interdisciplinary research for a successful approach.

Three examples of this "chemical synthetic biology" approach are given in Chapter 9. The first example deals with the synthesis of proteins that do not exist in nature, and dubbed as "the never born proteins" (NBPs). This research is related to the question why and how the protein structures existing in our world have been selected out, with the underlying question whether they have something very particular from the structural or thermodynamic point of view (for example, folding). The NBPs are produced in the laboratory by the modern molecular biology technique, the phage display, so as to produce a very large library of proteins having no homology with known proteins.

The second example of chemical synthetic biology has also to do with the laboratory synthesis of proteins, but, this time, adopting a prebiotic synthetic procedure, i.e., the fragment condensation of short peptides, where short means that they have a length that can be obtained by prebiotic methods.

The scheme is illustrated and discussed, being based on the fragment condensation catalyzed by peptides endowed with proteolitic activity. Selection during chain growth is determined by solubility under the contingent environmental conditions, i.e., the peptides which result insoluble are eliminated from further growth. The scheme is tested preliminarily with a synthetic chemical fragment-condensation method and brings to the synthesis of a 44-residueslong protein, which has no homology with known proteins, and which has a stable tertiary folding.

Finally, the third example, dubbed as "the minimal cell project". Here, the aim is to synthesize a cell model having the minimal and sufficient number of components to be defined as living. For this purpose, liposomes are used as shell membranes, and attempts are

made to introduce in the interior a minimal genome. Several groups all around the world are active in this field, and significant results have been obtained, which are reviewed in this article. For example, protein expression has been obtained inside liposomes, generally with the green fluorescent protein. The authors' last attempts are with a minimal genome consisting of 37 enzymes, a set which is able to express proteins using the ribosomal machinery.

These minimal cells are not yet capable of self-reproduction, and this and other shortcomings within the project are critically reviewed.