Robustness: confronting lessons from physics and biology

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ABSTRACT

The term robustness is encountered in very different scientific fields, from engineering and control theory to dynamical systems to biology. The main question addressed herein is whether the notion of robustness and its correlates (stability, resilience, self-organisation) developed in physics are relevant to biology, or whether specific extensions and novel frameworks are required to account for the robustness properties of living systems. To clarify this issue, the different meanings covered by this unique term are discussed; it is argued that they crucially depend on the kind of perturbations that a robust system should by definition withstand. Possible mechanisms underlying robust behaviours are examined, either encountered in all natural systems (symmetries, conservation laws, dynamic stability) or specific to biological systems (feedbacks and regulatory networks). Special attention is devoted to the (sometimes counterintuitive) interrelations between robustness and noise. A distinction between dynamic selection and natural selection in the establishment of a robust behaviour is underlined. It is finally argued that nested notions of robustness, relevant to different time scales and different levels of organisation, allow one to reconcile the seemingly contradictory requirements for robustness and adaptability in living systems.

Key words: robustness, stability, selection, noise, adaptability, canalisation, self-organisation, regulatory networks.

Contents

I. INTRODUCTION

The increasing demand for interdisciplinary approaches to tackle biological issues requires a common language and concepts. Such a shared understanding is clearly not met as regards the notion of robustness. At least, a consensus exists on the fact that discussion of robustness only makes sense if we specify what feature (Section II) and with respect to what class of perturbations this feature is robust (Section III). The main question addressed herein is whether the notion of robustness developed in physics is relevant to biological systems or whether a specific notion is required. Discussion will be substantiated with examples taken as benchmarks, for instance protein folding, chemotaxis, homeostasis, or gene expression and development (Section IV). Several general mechanisms can be identified (Section V) ranging from ubiquitous physical mechanisms (*e.g.* stability, universality, or self-organisation) to specifically biological ones (*e.g.* redundancy, repair or regulation).

Special attention will be given to the seemingly conflicting relation between robustness and noise (Section VI) often questioned in the biological literature. The relation is multiple; the notion of noise, like that of robustness, covers several meanings and instances: thermal noise, statistical fluctuations due to the finite size of the sample, spatial disorder encapsulated in a probabilistic description of the local parameters, or an uncontrolled external influence where our ignorance, like in the case of disorder, is accounted for in a random input term.

Another issue is the apparent tension between robustness and adaptability. It will be argued here that the tension can be resolved by considering adaptability as a higher level of robustness (Section VII). Achieving a proper compromise between robustness and chance for adaptation provides a strong evolutionary constraint, required for the long-term survival of the species in an ever-changing environment. As such, it offers a clue to understanding the organisation and regulation of living systems.

The basic definition according to which "a feature or phenomenon is robust with respect to the set of perturbations that it is able to withstand" will thus be gradually endowed with more precise and more quantitative (although possibly multiple) meanings, through examples, caveats and theoretical developments. Conclusions of this analysis (Section VIII) will delineate a hierarchy of context-dependent notions all deserving to be named 'robustness'.

II. ROBUSTNESS OF WHAT?

Speaking of the robustness of a system only makes sense after having defined those features whose persistence is under threat. It could be either a stationary state, the dynamic mechanism generating this stationary state, a regulatory scheme (*e.g.* topological features of a regulatory network), or a function of a living system, or a developmental pathway (that is, the sequence of 'choices' or 'decisions' made at each step in the making of an organism). This non-exhaustive list illustrates the wealth of different issues covered by the same term. Moreover, it appears relevant to distinguish the robustness of the process producing these features, corresponding to feature reproducibility, from the robustness of the features once established, namely feature persistence. It is here essential to supplement any assertion about robustness with the tolerance with which the feature reproducibility is to be appreciated and the time scale over which the feature persistence is to be observed. It is another issue, tackled in Section V, to unravel the possible mechanisms ensuring feature reproducibility (feature persistence) and in what respects, if any, the answer differs for physical and biological systems.

In contrast to robustness associated with feature persistence, resilience refers to the possibility that, after an instantaneous perturbation, the original behaviour is restored only after a transient in a markedly different state or regime On mathematical grounds, in the context of dynamical systems theory, the distinction between robustness and resilience is illustrated by the distinction between a stable fixed point and an excitable fixed point. In material sciences, resilience is the ability of an object to recover its original structure and shape after a deformation once the constraint causing the deformation is removed; it might exhibit some transient hysteresis or slow relaxation,

but the initial state is finally restored. Another example is the resilience of an ecosystem: the introduction of a new species, for instance, could lead at first to its proliferation accompanied by a dramatic decrease and seeming disappearance of some native species, before accommodation to the intruder takes place over a few generations, after which the original ecosystem may be almost restored; by contrast, robustness in this case would be the persistence of the original ecosystem with roughly the same population levels, continuously accommodating the intruder. We thus see that the difference between robustness and resilience is in practice not so deep, since increasing the tolerance and time scale of the observation can erase the difference and turn a resilient feature into a robust one.

III. ROBUSTNESS WITH RESPECT TO WHAT?

After having defined the object, namely the system and its observable features, speaking of the object's robustness only makes sense with respect to a specified set of perturbations: the system's state or behaviour should not experience any qualitative change and the variation of the relevant observables caused by perturbations of the set should be bounded and of relatively low amplitude. When it is possible (and tractable) to endow each perturbation of the set with its probability of occurrence (that is, turning the set of perturbations into what is called a statistical ensemble), the response of the system is described by the probability distribution of the change to some relevant observable. Robustness is then assessed by considering the width of this distribution. A narrow and centered distribution is associated with a robust situation since the observed behaviour is almost always very close to the unperturbed one. By contrast, power-law statistics accounts for a highly variable and unpredictable outcome, with a non-negligible probability of observing extreme events, totally different from the unperturbed behaviour, hence corresponding to a non-robust situation.

Regarding the possible ensembles of perturbations, it is important to distinguish robustness with respect to: (1) transient *versus* permanent perturbations; in the first case, it makes sense to simply investigate whether relaxation back to the unperturbed situation takes place, while the second instance necessarily implies some updating of the system behaviour, whose nature, strength and impact on the considered feature are precisely to be appreciated; (2) large *versus* small perturbations; the latter belong to linear response theory while the former take into account the complete and currently non-linear time evolution of the system; (3) changes in the system *versus* changes in its surroundings; (4) changes in the system parameters *versus* changes in its constitution (*e.g.* removal of a link or a node in a network, removal of part of an organ, introduction of a novel species to an ecosystem), the latter being obviously far more difficult to formalize and analyze than the former; (5) additive noise, when a stochastic forcing is added to the deterministic contributions ruling the dynamics of the system, and multiplicative noise, when noise enters the parameters of the dynamics. Indeed, because it modifies the evolution rates and/or the strength of couplings and non-linearities, multiplicative noise can have a qualitative effect on the observed behaviour, not reducing to a mere blurring of the system's evolution.

Beyond robustness with respect to a given class of perturbations, one has also to investigate, especially in a biological context, robustness to targeted, concerted or adapted perturbations, in particular, perturbations in multiple dimensions, of multiple sub-systems and several parameters (Jen, 2005). A configuration or regime might be viable within a large class of conditions but dramatically break down upon a specific perturbation or sequence of perturbations.

An additional criterion should not be ignored: the time and space scales of the perturbations, compared to the system's characteristic scales, generally have an impact on the observed response. We expect a very different response to fast perturbations, that self-average out before the system begins to respond, compared to slow perturbations of the surroundings, that the system follows adiabatically, relaxing to a stationary state between any observable change of external conditions (Lesne, 2006*c*; Castiglione *et al.*, 2008). It will be argued in Section VII that a such a distinction offers a clue to reconcile robustness and adaptability of the same feature.

IV. A FEW BASIC EXAMPLES

We start with a few paradigmatic examples, to provide benchmarks and illustrations in the general analyses and discussion that follow.

(1) Protein folding

Only a vanishingly small fraction of possible amino-acid sequences corresponds to functional proteins. Typically, a functional protein exhibits a primary folded structure, called its native structure, and several metastable ones; its function is fulfilled by involving transitions between these conformations. Proper functioning requires not only that the conformations are structurally robust but also that the transitions between them occur in a controlled way. Irreversible misfolding, as in prions, can lead to major health impairments or ageing (Söti & Csermely, 2006). The underlying property ensuring the robustness of both the protein conformations and its conformational transitions is a strongly featured free energy landscape (Nienhaus *et al.*, 1997). This landscape has been exquisitely tuned during evolution of the organism (and associated co-evolution of all its molecular constituents) so as to now exhibit the privileged pathways towards [the so-called 'folding funnel' (Onuchic *et al.*, 1995)] and between the relevant conformations, like valleys and passes between villages in a real landscape.

For several proteins, this basic channeling is supplemented with the presence of chaperones, that is, specific auxiliary proteins binding the misfolded ones and able to remedy their functional defects (Wilson, Yerbury & Poon, 2008). Other chaperones are involved in the folding process or in the assembly of macromolecular complexes. The very existence of chaperones and their functions also relies on the co-evolution that shaped the molecular constituents of cells and tissues.

Robustness here concerns both structures and structural transitions, with a direct impact on functional robustness. It follows basically from the channeling relief of the object's free energy landscape, supplemented in some cases with dedicated guiding mechanisms able to reinforce the free-energy channeling and repair mechanisms minimising the functional consequences of misfolding.

(2) Chemotaxis

Sensing by bacteria, as involved in their chemotactic behaviour, essentially incorporates thermal fluctuations. As macroscopic observers, we are sensitive to a huge number of events or molecules whose relative fluctuations at observation scales are negligible; in other words, we are sensitive to deterministic concentrations thanks to the integration achieved by our sensory cells and brain. By contrast, a bacterium has access only to a small number of molecules, and it cannot rely on differential sensing by comparing the numbers of nutrient molecules arriving respectively from the right and the left (Berg, 1986; Segall, Block & Berg 1896). Indeed, the gradient direction estimated in this way often yields incorrect information, because the relative magnitude of molecular fluctuations is large enough to invert the ordering of the average values. Nevertheless, it has faithfully to produce a determined outcome (namely a determined displacement at some mesoscopic or macroscopic scale) from these highly noisy pieces of knowledge; and actually it does (Mao, Cremer & Manson, 2003).

The solution that bacteria have evolved is an alternation of directed motion, when all the flagella of the bacterium act synergistically, and almost standing disordered motion, when the flagella exhibit no coordination in their movements, after which the directed motion resumes in a random direction (Berg & Brown, 1972); what is regulated by number sensing is the duration of the directed phases, that increases with the number of detected nutrient molecules. The net result emerges only at a higher scale, large (in time) compared to the duration of a phase and (in space) to the size of the corresponding step. This mechanism is able to buffer over the long term the impact of a few ill-directed local motions. At our scale, the bacterium behaviour is robust insofar as it achieves, at our scale, the properly oriented motion notwithstanding microscopic fluctuations.

On theoretical grounds, the features of the emerging motion at a large scale appear to follow directly from the strong law of large numbers for a biased random walk (a celebrated theorem

of probability theory) applied to the sequence of steps performed by a bacterium. This 'statistical mechanism' allows bacteria not only to manage with finite-size fluctuations and produce a directed motion at a large scale, but also to buffer the effect of local perturbations, for instance a local concentration of chemicals interfering with the nutrient detection, a local event turning the bacteria in the wrong direction or a transient dysfunction of bacterial motility. Their chemotactic behaviour is thus robust to local noise whatever its origin. An alternative explanation, based on the directional optimisation of the received quantity of information, has been recently proposed (Vergassola, Villermaux & Shraiman, 2007); robustness would follow from the existence of an underlying universal variational principle (see Section V.2).

Robustness here refers to the reproducible emergence of a deterministic component in the motion, despite its reliance on highly fluctuating elementary sensorimotor mechanisms; it appears as as a concrete consequence of statistical laws and limit theorems of probability theory.

(3) Internal temperature

Homeostasis, namely the ability of a living system to maintain internal variables such as concentrations, temperature or pH in a constant state despite ever-changing surroundings and inputs, is an ubiquitous instance of biological robustness. A familiar example is internal temperature. The first option for the organism is simply to let the physical law of thermal equilibrium govern its temperature, following the external temperature and its variations. The second option is to achieve a thermal regulation system able to maintain a fixed internal temperature (37° C for humans) whatever the external temperature is. This option is costly, both for building the regulatory system and for providing the free energy required for its functioning, but it considerably improves the efficiency of the metabolism. A related long-term mechanism is the adaptation of the reference temperature value so as to find the best compromise between the good metabolic efficiency allowed by this temperature and the energy cost of its maintenance.

Robustness here means the preservation of the 'internal milieu' [introduced by Claude Bernard around 1860, (Holmes, 1986)] by means of numerous coupled feedbacks and active regulatory circuits ensuring the global stability of the reference state.

(4) Self-organisation by means of self-reinforcement

Three examples will illustrate how a self-reinforcement mechanism leads to the emergence of a robust collective behaviour or pattern.

Most collective behaviours in ant colonies spontaneously arise by means of pheromone trails left by ants. Each ant generates such a trail, recognised by the following ants, when it walks towards a food source and back to the colony. Where there are two competing food sources, the shuttle of the ants will be faster between the colony and the closest one, hence the corresponding trail will be more traveled and reinforce faster. This mechanism ensures the persistent choice of the closest food source, with no need for supervised communication or centralised regulation. In case of equally distant sources, a minute fluctuation favouring visiting one source against the other will be at once enhanced, rapidly leading to the persistent choice (although initially random) of one of the sources. No additional mechanism is required to ensure the robustness of the behaviour once established (Beckers, Deneubourg & Goss, 1993).

Similar reinforcement of chemical trails can be invoked in self-organisation of microtubules: shortening of a microtubule end due to the disassembly of tubulin units leaves a trail of tubulin, that feeds the assembly of another microtubule and favours the alignment (treadmilling) of the microtubules, one following another. If moreover an external field (even a relatively weak one, such as gravity) is applied at a given critical stage of microtubule assembly from a tubulin solution, it triggers the coordination of the basic trailing interactions between microtubules, and the final outcome exhibits a macroscopic order. Once the critical stage is over, the macroscopic pattern is irreversibly established and quite insensitive to noise and perturbations; it thus corresponds to a robust organisation of microtubules, that can withstand various perturbing events or modifications

of the intracellular conditions. Such a self-organised assembly of microtubules has been observed *in vitro* and it offers a plausible mechanism contributing to cytoskeleton formation *in vivo* (Glade, Demongeot & Tabony, 2004).

A third example can be found in neurons. It is know as the Hebb rule and corresponds to an increase in the relative strength of the interaction between two neurons (that is, the weight of their oriented connection in neural network modeling) when it is successfully at work: this rule states that the weight of the connection rapidly becomes proportional to the activities of upwards and downwards neurons, hence it increases when the connection happens to contribute consistently to the global and emergent pattern of activity. Such adaptation of the connection weight is achieved concretely by self-reinforcement of synapses, ensured by self-organisation of receptors stimulated by the transmitted chemicals, leading to increased concentration of receptors under the active synapses (Choquet & Triller, 2003). This mechanism perpetuates the activity pattern by imprinting it in the neuron interaction network, making it insensitive to a moderate amount of noise. As such, the Hebb rule is the basic ingredient of memory and learning processes (Hopfield, 1982).

Robustness here refers to the reproducible emergence of a determined outcome, irreversible and unaffected by noise once established, by means of self-amplification of some initial feature.

(5) Hair-cell self-adaptation at a bifurcation point

A striking example of robust functioning in a non-generic but functionally optimal state has been demonstrated in the hair cells involved in hearing (Camalet *et al.*, 2000). The oscillatory dynamics of the hair-cell membrane and 'hairs' (the kinetocils) is coupled, *via* its role in opening ionic channels, to the ionic concentration dynamics. The latter in turn exerts a feedback on the control parameters of the membrane dynamics, making it possible to tune this dynamics near to the so-called Hopf bifurcation point, that is, just above the onset of oscillations. The interplay between the membrane dynamics and the ion concentrations is ATP-dependent, hence the self-adapted functioning of the hair cell is costly. But it is highly beneficial to the function of the hair-cell because functioning near a bifurcation point considerably enlarges the amplitude range of faithful detection: this interplay has been selected and settled in the course of evolution. Self-adaptation on a non-generic point (like a bifurcation or a threshold) stabilised and turned into a robust feature by a feedback loop, as illustrated by this example, has been argued to be a frequent feature of living systems (Thom, 1975; Kauffman, 1993).

Robustness here refers to a reproducible and persistent functioning of a subsystem in a specific state or regime, and it is achieved by means of feedbacks and regulatory circuits ensuring its global stability; a more fundamental underlying mechanism is evolution by natural selection, explaining how such feedbacks stabilising the system in a non-generic state or regime have settled.

(6) Information transfer and processing

Quantifying and improving robustness of information transfer in telecommunications was the primary motivation in the development of information theory (Shannon, 1948). Shannon showed that reliable transmission and processing of information in noisy channels requires redundancy, meaning here the communication of more bits than strictly required for transmitting the information content of the message (Tautz, 1992). Although those extra bits are costly to transmit and process, they allow one to check and correct a partially corrupted message. It can be proved that in order to preserve the information content, the average level of redundancy should be larger or equal to the average error rate. However, the extra bits corresponding to the minimal (or close to minimal) redundancy level are not simply repetition of part of the message: several crafty proofreading procedures have been devised to exploit dedicated extra bits of information into error-correcting codes (MacWilliams & Sloane, 1977).

The most prominent biological example of information transfer is provided by DNA replication. The original strand is methylated whereas the newly synthd one is not, so that specific repair enzymes (BER) can recognise the base responsible for a mismatch and replace it with the correct

one, properly complementing the native base lying on the template strand.

Robustness in information processing also relies on error avoidance. A simple and efficient mechanism enabling a dramatic decrease in error rate is that of double checking, encountered from the molecular level, [in DNA replication and transcription and mRNA translation (Hopfield, 1974) or molecular recognition such as of a knot in DNA by a topoisomerase (Yan, Magnasco & Marko, 2001)] to the macroscopic level (*e.g.* closure of a carnivorous plant once a fly as entered its calyx). A very low error rate is achieved by performing two successive and almost independent checks: the fly has to hit the walls of the flower calyx twice before it closes, the agreement between the DNA template and the nucleotide added to the newly synthesised strand undergoes a double proofreading, and so on. The overall error rate is thus roughly the square of the error rate achieved after a single check.

Robustness here means the faithful transmission or processing of information, *e.g.* genetic information, and it is ensured by various mechanisms (redundancy, proofreading, repair) allowing avoidance of errors or curing them.

(7) Cell differentiation

Cell differentiation, occurring during development or in response to a signal or an external stimulus, corresponds to a drastic change in the gene expression profile of the cell, with no underlying change in the DNA sequence; it is associated with numerous qualitative changes in the cell phenotype hence contrasting with the homeostasis otherwise characterising the cell states (Section IV.3). Two robustness issues are relevant: *(i)* the persistence of the cell type after it has appeared; namely, whether the new expression profile is durably (or irreversibly) established and whether it is transmitted to daughter cells during mitosis; *(ii)* the robustness of the cell differentiation process itself, namely whether it occurs at the proper place and time and leads to the proper cell type, either during embryogenesis so as to ensure the development of a viable and fit organism, or during adulthood enabling the proper response of the organism to some stress or environmental change. The question is to unravel the origin of each of these two experimentally well-assessed properties, and to appreciate the respective roles of genetic information, epigenetic memory and regulation, initial conditions, surroundings and boundary conditions in their underlying mechanisms.

The observation that cells sharing the same genome might exhibit different phenotypes underscores the now acknowledged fact that the genome does not prescribe alone all aspects of the functional organisation of the cell. For instance, cell morphology, metabolism, membrane potential and motility are neither simply nor exclusively ruled by the genomic sequences. Hence it is not enough to invoke the stable inheritance (if any) of genetic information to explain the reproducible and coordinated appearance of well-defined cell types, their robust spatial localisation during development and their persistence during the organisms life. Major epigenetic regulatory events should be invoked to explain both cell differentiation and cell type persistence. It is now currently argued that a second level of code, superimposed on the first-level genetic code, controls cell epigenetic modifications; this code is likely located at the chromatin level, involving post-translational modification of the histone tails (Turner, 2000; Jenuwein & Allis, 2001) articulated into complex regulatory networks (Benecke, 2003; Benecke, 2006; Lesne, 2006*d*).

Another viewpoint considers as a working hypothesis that cells differentiate at random (at a higher rate when they experience stresses, such as starvation) and that the cell types that are the fittest in the given context survive, self-organise and segregate in different tissues. Competition, mutual selection or co-evolution are at work and claimed to be sufficient, without any regulatory program or signaling, to account for the cell differentiation occurring during development and the resulting cell-type distribution within an organism (Stockholm *et al.*, 2007).

These two views appear to be complementary rather than mutually exclusive. The former is able to guarantee the random variety of cell types on which the latter relies, then to ensure the persistence of the successful types through epigenetic or dynamic memory. We moreover underline that differentiation process will rely on the biochemical, physical and topological properties of the chromatin fibre, fine-tuned during evolution, for generating different patterns of gene expression, in either a random or regulated way (Lesne & Victor, 2006; Lesne, 2006*d*).

In the context of cell differentiation, robustness can be addressed either about the outcome (persistent cell types) or about the process (differentiation program, patterning), with no obvious relation between the respective answers. Several mechanisms can be invoked and questioned, ranging from deterministic specialisation of the cell to epigenetic stabilisation of stochastic changes in the cell expression profile.

(8) Developmental robustness

As regards developmental robustness, three observed facts require explanation: *(i)* the faithfulness in reproducing in each new-born organism the features of the species; *(ii)* the faithfulness in reproducing specific features of its parents; *(iii)* the faithfulness in maintaining the organism during the course of its life (or, in insects or amphibians, in passing successfully through metamorphosis leading to the adult form). In other words, one has to explain why and how an organism looks like any other one of the same species, like its parents and like itself all through its life. These three facts are central to the foundations of evolution theory: selecting species and individual phenotypes and performances amounts to selecting lineages, that is, genotypes.

Developmental robustness was first investigated by looking for concrete and heritable entities whose robustness would be sufficient to ensure the robustness of the phenotype; DNA sequences were the first natural candidates. But this viewpoint is confronted by two caveats: having a robust underlying entity is neither sufficient nor necessary to ensure the robustness of the resulting phenotype, and DNA is not, in itself, robust but prone to mutations and epigenetic changes.

As in the related case of cell differentiation (see Section IV.7) we are led to reexamine the role of genes in the accuracy and faithfulness of development. A major role is presumably played by several regulatory networks, devoted to buffering external or internal variability and perturbations so that they impact in a filtered and regulated way on gene expression (De Jong, 2002; Benecke, 2006). A second layer of complex regulation might function, after transcription, to buffer the influence of mutants or ill-adapted proteins, by exploiting redundancy and feedback control abilities of protein-protein interaction networks and metabolic networks (Edwards & Palsson, 2000). This point has been partly underlined and discussed by Waddington (1940) whose concept of canalisation, describing qualitatively the buffering of variability and perturbations during development, has now been substantiated with concrete mechanisms (Kerszberg, 2004; Horstein & Shomron, 2006).

Failures in achieving proper development happen either because the mutations or the perturbations are individually too deleterious, or because they occur in a network specially sensitive to their influence and trigger a bifurcation of its dynamics. The systematic analysis of developmental abnormalities (teratology) and their targeted induction [in the spirit of the seminal experiments by Hans Spemann around 1924, (Hamburger, 1988)] are a privileged means to identify genes that are essential and critical developmental stages in which robustness is weaker and can be challenged by some specific mutation or perturbation.

In the context of development, robustness can be addressed either about the outcome (viable organism) or about the process (controlled and reproducible developmental stages). Several mechanisms can be invoked and questioned, ranging from physico-chemical principles of pattern formation to cell collective behaviour to genetic control, epigenetic memory and selection at the cell level, presumably each playing a part in development and its robustness.

V. MECHANISMS

General mechanisms that account for various instances of robustness are identified, below, without any claim of exhaustiveness. Most have been encountered in the above examples, which showed that different ingredients might contribute to several different robustness properties. These mechanisms range from physical ones, that can be found in both inanimate and living systems to specifically biological ones that indirectly or even directly rely on natural selection.

(1) Conservation laws, symmetries and symmetry breaking

(a) Conservation laws

Any mechanism that reduces the set of accessible states of a system will contribute to its robustness, merely in limiting its possible behaviours; for instance, resource limitation obviously prevents unbounded growth, without the need for negative feedback. Such limitations are clearly not sufficient alone to explain the robust features of a complex adaptive system, since any change in the constraints will have a direct repercussion on the observed behaviour. They might nevertheless contribute significantly if the constraints follow from an inescapable conservation law or if in turn some regulatory mechanism controls these constraints. The robustness issue shifts from that of the system to that of the constraints). Hence they should be clearly identified in the preliminary stage of the analysis.

Conservation laws confine the system to a lower dimensional hence non generic subset of the original phase space, where the phase space of a system is the space in which its state is described and represented (the space of positions and velocities for a moving point mass, or the space of concentration values for a chemical system) For instance, the micro-canonical ensemble $E = const.$, where E is the system total energy, would not be robust in the absence of energy conservation law, that enforces the energy value to be exactly equal to E instead of generically vary into a neighborhood of E. Conservation laws cannot be violated except transiently, in out-of-equilibrium systems, where it is possible to have a transient accumulation of charge or matter in the system at cost of a transient imbalance between the entering and exiting fluxes, or through local violations, in segregated domains, that globally balance each other.

Energy, matter or charge conservation laws are essential constraints but they can accommodate so many configurations and transformations of the system that they only weakly enforce the robustness of its detailed molecular structure and dynamics. For instance, charge neutrality of a complex molecule is a very stringent constraint, but it is not constructive insofar as a huge number of conformations satisfy this constraint. In particular, it cannot on its own explain the robustness of protein folding (Section IV.1), although it considerably influences the protein energy landscape relief and the possible paths towards the native structure of the protein, in an inescapable way.

By contrast, topological constraints, like the linking number conservation of an elastic rod (*e.g.* DNA, chromatin fibre or actin filaments), are not only stringent constraints but also constructive ones, having a strong and direct impact on the conformation and dynamics of the system (Mozziconacci *et al.*, 2006 [The linking number of a ribbon (or an elastic rod) is the number of turns imposed at one end of the ribbon before anchoring the two ends or gluing them together to form a closed loop (in the latter case, the linking number takes only integer values). It is an invariant quantity that can be modified only by cutting the ribbon and changing the number of stored turns before closing it again (Crick, 1976).]. For instance, these constraints are involved in the formation of supercoiled structures called plectonemes (frequent in old telephone wires) and they turn a local change in the rod structure into a long-range effect, *e.g.* unfolding of the whole constrained rod. The closely related symmetry constraints, associated for instance with the requirement for a regular folding in a protein or macromolecular assembly (*e.g.* haemoglobine or nucleosome arrays) are implemented through elastic constraints or more general mechanical constraints.

Organisation, architecture, geometry, symmetries and topological constraints generate a channeling of the dynamics and associated equilibrium (or stationary states) that ensures their reproducibility.

(b) Symmetry arguments in pattern formation

Let us underline the dual status of symmetry arguments as regards robustness, *e.g.* of a shape. On the one hand, pattern formation necessarily involves some symmetry breaking from the initially featureless homogeneous substrate: think of the inspiring example (Karsenti, 2008) of the periodic array of convection cells that spontaneously arises in a layer of viscous fluid heated from below (the celebrated Rayleigh-Bénard experiment). Information storage or memory in artificial or living systems similarly involves some symmetry breaking (Leyton, 2001). Reproducibility of the symmetry breaking process is required to ensure that of the emerging structure. But on the

other hand, the remaining symmetries of the structure play an important role in its persistence: a symmetric equilibrium structure in general achieves a local minimum of free energy (thanks to stacking interactions, for instance) so that any smooth departure from the symmetric state will be energetically prohibited. Symmetric structures are thus expected to be robust with respect to small perturbations. Similar arguments hold in symmetric far-from-equilibrium structures, but now relying on some flux optimisation criterion.

Symmetry and symmetry breaking arguments are thus essential in several instances of reproducible morphogenesis and formation of robust patterns.

(c) Compartmentation

A special instance of symmetry breaking is space compartmentation, either by membranes or dynamic segregation generating self-organised compartments. It has a direct impact upon robustness of the various phenomena that take place in the system, in preventing mixing of different inputs, signals or reactive elements. As such, it has been often selected in biological systems and it is observed for instance in living cells (a prominent example being the nucleus of eukaryotic cells). We here suggest extension of this idea that robustness can be promoted simply by segregating the relevant processes from the perturbations, and consideration of various instances of generalised compartmentation, either *(i)* in time: synchronisation within subsystems (as in neural networks), oscillations (for example those exhibited by metabolic reactions), processive events interspersed with pauses (as in polymerase activity); *(ii)* in nature: for instance conformational transitions that differ in their driving force, caused either by free-energy minimisation, chemical reactions, or elastic stresses; *(iii)* in rates, for instance rate-based discrimination between competing reactions; or *(iv)* in scales, when scale separation allows decoupling of slow and fast variables. Any effect of a given of a set of perturbations is here prevented in a built-in way because these perturbations cannot interfere with the system or process of interest.

(d) Digital encoding

Another instance where restricting the set of possible states promotes robustness is encountered when the system is bound to lie in a few predetermined discrete states, with a negligible probability of being observed between them. Such a robustness is observed in excitable media: for action potentials propagating along an axon, their shape is fully prescribed by the excitable dynamics of the axon membrane potential, allowing us to reduce them to 'spikes'; their triggering depends on the presence of some excitatory input, whose amplitude has no influence provided it overwhelms a given threshold. This robust spiking behaviour of neurons achieves a binary digitisation of the nerve impulse. The binary encoding attenuates the propagation of errors, hence ensuring the reliability of neurocomputing, exactly as in electronic computing.

Binary encoding is also encountered in switches, that is, systems which a positive feedback loop drives into one of two markedly different equilibrium or stationary states (Thomas, 1998). Switches are ubiquitous in biological systems, and it has recently been proposed in (Brandman *et al.*, 2005) that the coupling of two feedback loops with different characteristic times ensures the sensitivity and the robustness of the switch.

Another familiar biological example of a signal written with a discrete alphabet is DNA and the genetic code. It shows that while any progressive and continuous degradation of the information by accumulation of infinitesimal perturbations or small amounts of noise are prevented, finite errors reflected in the modification of a symbol into an erroneous one can occur. But the discrete nature and finite number of states allows the establishment of proofreading and repair mechanisms (see Section IV.6) that would not be possible for a continuum of states.

(2) Variational and optimisation problems

A large class of systems, in physics, ecology or economy, can be described as optimisation problems, by recasting their observed state or behaviour x as the minimum of a function $U(x)$ like free energy, inverse fitness or cost function. The paradigm is that of a landscape (Sherrington, 1997; Wales, 2003) endowed with a gradient dynamics (following the steepest descent like water on a real landscape where $U(x)$ is the altitude at point x, described by its latitude and longitude) and

perturbed by some noise. This noise originates from thermal noise in case of the energy or free energy landscape of a molecular complex; it corresponds more generally to an external incoherent influence, allowing the system to jump across barriers and move transiently against the gradient. The energy landscape is defined over the space of microscopic states of the system, as a function of all its microscopic degrees of freedom. At a mesoscopic level, the system state is described by only a few collective variables; the relevant landscape is the free energy landscape, accounting for the entropic contribution of the microscopic degrees of freedom underlying each of these collective variables (hence depending on temperature) (Wales, 2003).

The constraints experienced by the system shape the relief of the landscape and delineate more or less accessible regions. Within this landscape paradigm, a quantitative appreciation of the robustness of a given state (either the optimal state or any local minimum) follows from the relief characteristics in the neighbourhood of this state. The direction associated with the largest slope is the less sensitive, since a strong increase of the landscape altitude $U(x)$, requiring a strong perturbation, corresponds to only a minor shift of the system state x in the underlying state space. A different robustness property is that of convergence towards the optimal state: it will be more robust the less rugged the landscape is, so as to avoid trapping in local minima.

The impact of noise is here dual: noise has to be low enough for the system to be trapped and remain in the optimal state, while some level of noise is required for the system to escape from transient trapping in suboptimal states. This effect is exploited in a numerical optimisation algorithm called simulated annealing: the transient addition to the gradient dynamics of a tunable amount of noise (corresponding to increasing then abruptly decreasing the temperature in a statistical mechanics context) greatly improves the exploration of the landscape, since noise provides energy to escape spurious local minima and allows finding the state of lowest energy, while annealing stabilises this latter state once found. We see here a counterintuitive effect of noise, that ensures the robustness of the convergence to the optimal state, despite the fact that the route followed by the system strongly depends on initial conditions and realisation of the noise, while the robustness of the optimal state is ensured by the landscape relief.

Such an effect of noise contradicts the general idea that noise hampers robustness; it shows that their relationship deserves a finer analysis, proposed in Section VI.

(3) Dynamic stability

Dynamic stability refers to several instances, differing in the entity that experiences a perturbation, either the initial or current state of the system, the parameters of its evolution law (where here evolution refers simply to variation of the system's state with time, with no reference to Darwinian evolution) or the expression of the evolution law itself, accounting for different or additional contributions. They correspond to three essential notions of dynamical systems theory: attractors, bifurcations, and structural stability. We shall investigate how biological systems challenge these notions.

(a) Linear and global stability of an attractor

The basic notion, simply termed stability, means that the system state is a stable equilibrium, or more generally a stable regime of the dynamics, in the sense that it is recovered if some bounded perturbation is applied at a given instant and shifts the system state to a perturbed one. Except in the trivial case of linear dynamics, one has to distinguish linear stability and global stability. For simplicity, these notions will be introduced in the simplest case of an equilibrium point. Linear stability expresses the relaxation to zero of infinitesimal perturbations of the equilibrium point in the phase space (that is, the space in which the system state is represented by a point, and its time evolution by a trajectory). For deterministic dynamical systems, it is assessed by linearising the dynamics around the equilibrium point: the eigenvalues of this linear evolution should all correspond to damping, that is, have a strictly negative real part. [This statement applies to dynamical systems, where the time variable varies continuously. In case of a dynamical description in terms of a discrete time variable, the fixed point is linearly stable if and only if all the eigenvalues of the linearised evolution have a modulus smaller than 1; the relaxation times are then given by the logarithm of the inverse moduli.] These real parts (more precisely the inverse of their

absolute values) are each related to the relaxation time of the perturbation component along the corresponding eigenvector. In practice, such a damped linear response ensures stability with respect to very small perturbations of the equilibrium state.

By contrast, global stability fully takes into account the non-linearities of the evolution law; it corresponds in practice to the relaxation to zero of large perturbations of the equilibrium state. Assessing global stability amounts to delineating a whole region around the fixed point, called its basin of attraction, such that any trajectory starting in this region ultimately evolves toward this fixed point. Global stability is far harder to prove than linear stability, in particular because there is no systematic method that would apply in all cases. A computationally heavy and still approximate approach is the determination of the border-lines separating two basins of attraction by an exhaustive numerical scanning of the evolution of a grid of initial conditions spanning the phase space. A more elegant and rigorous way is to evidence by an educated guess a positive function, named a Lyapounov function, that is strictly decreasing along the trajectories and reaches its minimum value 0 at (and only at) the fixed point; but such a function does not always exist, and even if it exists, it is not always easy to find.

On mechanistic grounds, global stability results from the dissipative nature of the dynamics, ensuring that the dynamics at long time scales is ruled by an attractor, namely a small region of the phase space where the system trajectory will ultimately lie. The attractor might be an equilibrium point, as in the simplest case discussed above, or a limit cycle associated with an oscillatory regime, or a more complicated set called a strange attractor in the case of chaotic dynamics. The acknowledged sensitivity to initial conditions of chaotic dynamics does not spoil the global stability of its attractor: trajectories could strongly depart one from another while all converging to the attractor in their own way; the sensitivity to initial conditions besides applies to trajectories embedded in the attractor and contributes to its 'strangeness' and intricate fractal geometry. Stability and robustness issues in conservative dynamical systems, namely systems in which the total energy is conserved, are central in physics (for instance, investigating whether the solar system is stable and robust to perturbation). They have a long and celebrated history, from Kepler to Newton to Poincaré and more recently Kolmogorov, Arnold and Moser whose KAM theorem expresses the persistence of regular trajectories as a function of the amplitude of the perturbation (Castiglione *et al.*, 2008). But they are totally irrelevant in biology since all living systems are open systems, and their dynamics (either chemical kinetics, oriented motion of motor proteins, cell motility, or population dynamics) all belong to the class of dissipative systems.

(b) Marginal stability and self-organised criticality

An empirical criterion accounting for robust pattern formation when several solutions are possible is that of marginal stability. It means that a unique behaviour is spontaneously and reproducibly selected, corresponding to the least stable solution among the dynamically stable solutions (Van Saarloos, 1987). For instance, in reaction-diffusion systems exhibiting a continuum of possible wave-front solutions (that is, solutions that propagate at a constant speed with a fixed shape), the actually observed front is the least stable one, that happens to be the slowest and steepest stable one. On the one hand, any slower front would be destabilised since minute fluctuations or perturbations in its shape are exponentially amplified and induce a breakdown of the solution; on the other hand, it can be shown that an interplay between the local slope of the front and its propagation speed (steeper for slower speeds) slows down the faster and flatter solutions and progressively rebuilds and restores the marginally stable solution (Lemarchand, Lesne & Mareschal, 1995). This marginal solution appears as the attractor of the global dynamics, hence as a robust solution.

Another instance of marginal stability is observed in so-called self-organised critical systems (Bak, 1996). The paradigmatic example is a sand pile, slowly fed from above by a falling current of sand and exhibiting at the same time a reproducible shape and slope, and unpredictable avalanches of all sizes and durations distributed according a power law. The globally stable regime (an attractor of the complete dynamics) appears to be locally a marginally stable configuration when considering as a subsystem a small region of the pile side.

In biological systems, we are often faced with more complex situations where marginal stability (and the ensuing robustness) follows from a feedback loop between the essential variables and

several well-tuned and mutually adapted parameters, as in the hair-cell example in Section IV.5.

(c) Structural stability, bifurcations and normal forms

Another notion, termed structural stability, means that the asymptotic regime remains qualitatively unchanged upon enough small perturbations of the dynamics itself. In mathematical terms, it means the flows of the original and perturbed systems are then smoothly conjugated (Guckenheimer & Holmes, 1983). It fails at bifurcation points, where a minute change in a control parameter across a critical value (called a bifurcation value) has qualitative consequences on the asymptotic regime, for instance changing the observed equilibrium point into another one or into an oscillatory regime; the transition observed at a bifurcation point is possibly discontinuous (what is called a sub-critical bifurcation). Bifurcation theory thus explains how a continuous variation of a parameter (*e.g.* an enzymatic rate continuously evolving with the enzyme sequence) might trigger a discontinuous event, like a qualitative change in the phenotype of an organism, hence offering a way to reconcile the gradual and discontinuous views of evolution. An important mathematical result is the the fact that the dynamics near a bifurcation point is essentially determined (through a smooth conjugacy) by the bifurcating behaviour of a minimal and universal model, called a normal form (Gang & Haken, 1989; Haken, 1996). It shows that only the marginal directions (that become unstable at the bifurcation point) matter in the bifurcating behaviour and control the changes observed in the attractor. A related notion is that of catastrophe, describing the topological types of singularities that might arise in the dependence of the asymptotic state with respect to the control parameters (Thom, 1975). Compared to bifurcations, catastrophes are limited to fixed points of gradient dynamics but with no restriction on the number of control parameters. These results about the genericity of bifurcations and catastrophes, as well as the evidence of universal scenarios (that is, determined sequences of bifurcations) leading to chaos show that some higher level of robustness is recovered in the way robustness to parameter changes is lost.

We have introduced above several notions of stability (linear, global, structural) for an attractor. Another interesting instance is the robustness with respect to a perturbation of the dynamics, or more specifically with respect to noise, of the coexistence of several attractors and associated multi-stability. Coexistence of attractors has a wide functional interest in biology. For instance, a generic sub-critical bifurcation associated with an 'S-shaped' bifurcation diagram, provides a switch mechanism between the lower and upper states, that is robust insofar as the transition to the upper state occurs at a larger value of the control parameter or stimulus amplitude than the reverse transition; the robustness will be stronger the wider is the distance between the knees of the S-shaped curve, that is between the two transition thresholds.

(4) Feedbacks loops

The persistence of a state occurs through different mechanisms at equilibrium and out of equilibrium. At equilibrium, it is passively ensured by energy barriers isolating the equilibrium state, preventing small perturbations and low-amplitude noise from having a qualitative effect: they only induce fluctuations around this fundamental state. On the contrary, for a non-equilibrium stationary state, the persistence is actively ensured by feedback loops and trade-offs between competing trends: such persistence is costly and observed only in open systems fed with matter and energy.

In a simple feedback loop, what is essential as regards robustness is the sign of this loop, that is, whether it contains an odd (negative loop) or even (positive loop) number of inhibitory relations (Thomas, 1998). A positive loop amplifies minute fluctuations around a reference state and induces robust multi-stability (Demongeot, Kaufman & Thomas, 2000). On the contrary, a negative loop is self-stabilising hence associated with homeostasis. Acknowledged examples are a servo-control mechanism in artificial systems (of either sign), an auto-catalytic reaction (positive loop) or a self-inhibited gene when the protein it encodes inhibits its transcription. More complicated and intermingled feedback loops arise in networks, *e.g.* metabolic or gene networks; the huge combinatorics of competing or synergistic loops allows fine-tuned and adaptive control, opening a whole field of investigations, namely regulatory networks (Section V.5).

In complex systems, feedbacks moreover 'cross the scales' insofar as the emergent features influence back the behaviour of the elements. An acknowledged situation is provided by a ferromagnetic

spin-lattice far below the Curie temperature. The influence on a given spin s_i of its interactions with all its neighbours, in other words the local field generated in s_i by all the other spins, can be approximated by the influence of a 'mean-field' supplementing the external field, if any, and depending only on the overall magnetisation; this feedback explains the emergence of a spontaneous magnetisation (in the absence of any external magnetic field). The feedback of the macroscopic state onto the microscopic elements can have a dramatic influence on the overall behaviour if it is able to induce a bifurcation of the elementary dynamics.

A more complex situation is encountered when an additional feedback couples the emergent features and the control parameters, and tunes these parameters to special values, as in the hair-cell dynamics (Camalet et al., 2000) presented in Section IV.5 or, in the above example, if not only the spin orientations but also the very value of the spin or the temperature were affected by the statistical features of the overall behaviour (Sornette, 1992). Such strong interlevel feedbacks are often encountered in living systems (originating from the co-evolution of the different levels of organisation) and endow them with the ability to control both their microscopic and macroscopic dynamics so as to settle in a non-generic but functionally beneficial regime. What becomes relevant for robustness is the efficiency of the overall regulatory scheme.

Let us finally underline that one might distinguish two kinds of feedbacks and regulatory mechanisms in biological systems: mechanisms that determine the state and those ensuring its robustness in an ever-changing environment. Mechanisms of the first kind already exist in physical systems (self-consistent fields in magnets and polymer melts, hydrodynamic feedbacks in sand dunes or coastlines). Mechanisms of the second kind are rather specific to biological (or artificial) systems.

(5) Network structure and redundancy

The robustness of a network is a shortcut for referring to the robustness of the phenomenon that the network description intends to capture, hence different instances and accordingly different mechanisms can be put forward. Only those where the network structure is directly involved in the mechanisms ensuring the robustness will be considered here.

A network is any system whose definition involves not only a set of elements (the nodes of the network) but also pairwise couplings or interrelations among these elements (the edges or links of the network). Let us cite for instance communication networks (where the nodes are cities and links are roads or airlines), social networks, or protein networks where a link between two proteins means that they have a significant mutual affinity.

Several paradigmatic models have been introduced, providing landmarks that pave the way towards a better understanding of network structure and dynamics (Lesne, 2006*b*): percolation (Stauffer, 1985), spin lattices and spin glasses (Amit, Gutfreund & Sompolinski, 1985), neural networks (Hopfield, 1982), coupled map lattices (Kaneko & Tsuda, 2001), or random graphs (Erdös & Rényi, 1960) and the fashionable power-law networks (Albert & Barabasi, 2001) [*power-law networks* are characterized by a degree distribution $P(k) \sim k^{-\gamma}$ at least for enough large degree values (where the degree k of a node is the number of its direct links with other nodes); they are a typical instance of *scale-free networks*, characterized by a broad degree distribution that reflects the heterogeneousness of the nodes and the absence of any characteristic degree]. The network structure is formally defined by a family of elements, the nodes (spins, cells, neurons, oscillators) and a family of links between these elements; various dynamics can then be envisioned, of three different kinds: growing or rewiring of the network, propagation or spreading in the network, or coupled node dynamics. Two main instances of robustness involving the network structure of the system can be considered:

(i) robustness of the connectedness. This is the issue addressed by percolation theory in the case of unconstrained and independent random wiring of the different links. The network is fully prescribed by the probability p that a link is wired among an admissible pair of nodes (either any pair of nearest neighbours in the case of standard bond percolation on a regular lattice, or any pair of nodes in the case of so-called random graphs). Equivalently, links are removed with a probability $1 - p$ from a configuration where all admissible pairs of nodes are connected. This latter procedure has been extended to random or ordered removal of nodes, the issue being to determine the average fraction that should be removed in order to fragment the network. It has

been shown that a power-law network is robust to failures but fragile with respect to attacks targeted to the most connected nodes, whereas these two ways of damaging do not have markedly different consequences in random graphs (Pastor-Satorras & Vespignani, 2004);

(ii) robustness of the dynamics, describing for instance some transport phenomenon in the network or the coupled evolution of the node states. An issue is to assess the persistence of the large-scale behaviour and properties (fluxes across the network, synchronisation, patterns of activity) after some local or transient perturbation; another one is to unravel the persistence of some localised outcome despite a global change; a third is to explain the robustness of the information transfer between two nodes or two regions of the network despite the presence of noise or perturbations. Among the main insights, it has been evidenced that networks currently exhibit redundancy of nodes and links that provides a way to circumvent local perturbations or failures such that they have no consequences on the overall dynamics. Obviously a communication network will be protected against the failure of a channel if diversions exist. In mice in which a specific gene has been knocked out, the expected strong functional consequences of the knock-out are often not observed, showing that the gene network exhibits either gene redundancy or dynamic adaptation, namely its regulatory dynamics is able to reorganise the node activity so as to compensate for the missing gene (Tautz, 1992). Another property of networked systems that contributes to their functional robustness is their plasticity, namely the possibility of rewiring or re-weighting the role of some connections; it enables the system to cope with novel inputs or changes in the node features. In several failure instances, the system viability might be restored by a change in connection patterns and weights. Numerous cases have been observed in locally damaged brains. This discussion illustrates the ability of networks to exhibit functional robustness despite their elements and elementary processes being not themselves robust. It is still an open question to account fully for the response of network dynamics to a perturbation, and how it could be either amplified or buffered, propagated of localised, according to the topology of the connections and elementary features (Bornhold & Schuster, 2002; Müller-Linow, Marr & Hütt, 2006; Lesne, 2007).

On experimental grounds, a current approach to probe the functioning of a system is to apply a targeted perturbation but it is not in general easy to interpret the result in case of a network, as illustrated by the gene knock-out example. A linear stability analysis of the perturbation effect (Grimbs et al., 2007) is often irrelevant, due to strong enhancement or on the contrary buffering both achievable by the feedback loops embedded in the network. To understand the internal mechanisms controlling the observed outcome, it is not enough to vary the inputs nor the environment. Neither is performing a 'site-directed mutagenesis' by changing elementary pieces and investigating the impact on the emerging behaviour, but again the interpretation is not straightforward. In fact, the very robustness of the network makes it difficult to unravel its structure and dynamics from the observation of the response to a perturbation, either local or global. For instance, in the case of a gene knock-out, observing a change in the biological function after the perturbation only means that the gene is somehow involved, either directly in the function or possibly very remotely in the regulatory network of this function (for instance ruling the synthesis of an auxiliary but ultimately influential ingredient insofar as it inhibits an otherwise competing pathway). Another experimental access is to record simultaneously the state or activity of all nodes, as is done in gene networks by means of high-throughput micro-array experiments. Whereas such experiments allow a direct reconstruction of the correlation network, inferring the actual couplings and interactions is far more difficult, most often offering several plausible reconstructed patterns of connections, showing again that network robustness hinders its experimental identification and understanding.

Finally, note that in living systems, complex regulatory networks are essential to functional robustness but usually not to minimal functionality: they are required mainly for guaranteeing the robustness of functions that could be achieved with far less ingredients and according to a far simpler scheme, as shown for instance by their implementation in older and simpler organisms (Carlson & Doyle, 2002). Moreover, regulatory networks contribute to functional robustness of biological systems in two opposite ways: either in decoupling the internal state of the system from its environment and ensuring its homeostasis despite the ever-changing influence of the surroundings, or in emulating the proper adaptation of the internal state so as to cope best with the environmental changes while preserving the biological functions. This point raises another question: understand-

ing the determinants leading an organism to withstand or to adapt to a perturbation, and some clues will be proposed in Section VII. In any case, robustness of the biological functions follows from the robustness of these regulatory networks and their dynamics. Conversely, failures in the regulatory networks and bifurcations of their dynamics (due for instance to environmental factors and stresses) can be tracked as a possible origin of ageing (Gavrilov & Gavrilova, 2001) and chronic diseases (Victor *et al.*, 2008).

VI. RELATIONS BETWEEN ROBUSTNESS AND STOCHASTICITY

Stochasticity is currently thought to conflict with robustness in three respects: *(i)* a fluctuating phenomenon is not robust; *(ii)* external noise might spoil the system behaviour; *(iii)* a stochastic process cannot produce a robust outcome. We shall examine these statements and argue that in some cases, they depart quite strongly from the observed behaviour; we shall see that stochasticity might even play a constructive role towards robust behaviours, particularly in biological systems.

(1) Statistical descriptions

To appreciate better in what respect stochasticity may or may not challenge the robustness of a system, let us recall a few basic notions and discuss the rationales underlying statistical descriptions.

(a) The notion of fluctuation

To be specific, the ordinary meaning of fluctuation (used to designate any non-monotonous variation in time, not necessarily random nor even irregular since the term 'periodic fluctuations' is often heard) has to be restricted to its statistical definition relative to a random variable X [The meaning of random ranges from "non deterministic" (as in "random variable") to a term referring to a "stochastic event or process without any correlation"; we shall use "fully random" in the latter case.]. The fluctuation δX is what remains once the deterministic part associated with the statistical average $\langle X\rangle$ has been removed, namely $\delta X = X - \langle X\rangle$. By definition, $\langle \delta X\rangle = 0$ and $\langle \delta X^2\rangle \equiv \sigma^2$ is the variance of X. The dimensionless ratio $\sigma/\langle X\rangle$ is currently used as a measure of the fluctuation strength, in other words the degree of randomness. The measure $\sigma/\langle X\rangle$ is relevant only if the distribution is a single narrow peak, in which case $\langle X\rangle$ coincides with the location of the peak and gives the typical value of X, while the probability of observing values $|\delta X|$ larger than a few σ is negligible so that σ gives the typical size of δX. In this case, the random variable X can be considered deterministic if σ is smaller than the characteristic scales of the observables depending on X, that is, the scales at which the phenomenon is perceived or has an impact, or the sensitivity with which it in turn exerts an influence. For instance, fluctuations in the concentration X of some reactant have no consequences if their standard deviation σ is far smaller than the characteristic scale of variation of the production rate $V(X)$, namely $|V(X) - V(X+\sigma)| \,/\, |V(X)| \ll 1$.

By contrast, the average $\langle X\rangle$ is rather meaningless for broad distributions, *e.g.* power-law distributions, for which there is no typical value for X and very large fluctuations are not negligible (σ might even be infinite in this case). Also in the case of multi-modal distributions, the average $\langle X\rangle$ does not describe the most probable value of X but results from cancellations between the typical values corresponding to the different peaks of the distribution; hence σ does not account for the fluctuations of X around its typical values but rather for the distance between these typical values. To describe more precisely the randomness without reconstructing the whole distribution, one often computes the higher moments, in particular the skewness $\langle \delta X^3\rangle$, measuring the asymmetry of the distribution, and the kurtosis $\langle \delta X^4\rangle - 3\langle \delta X^2\rangle^2$, allowing to detect quantitatively the presence of heavy-tailed or multi-modal distributions.

(b) Epistemic status of noise and stochasticity

Whereas a fluctuation refers to the observed randomness of a given quantitative feature of the system, noise refers to a seemingly stochastic influence experienced by the system under consid-

eration, typically an uncontrolled and highly fluctuating influence depending on so many factors, some being unknown, that it is hopeless to describe it except in a probabilistic setting. The apparent randomness of noise thus currently comes from the large number of microscopic degrees of freedom it involves, that are not explicitly described; but the evolution of each of these degrees of freedom might well be deterministic at the microscopic scale, showing that there is not a difference of nature between noise and a deterministic perturbation. The distinction between stochastic and deterministic dynamics, often viewed as an intrinsic and unambiguous feature, is rather an apparent feature and operational modeling choice. Chaos theory provided an additional support of this claim, since it has been shown that a chaotic dynamics, although purely deterministic, typically produces an erratic trajectory, exhibiting the same random features as some random process when observed at large enough time scales. In fact, there is an exact conjugacy (one-to-one relation) between the trajectories generated in [0,1] by the chaotic map $x \to 4x(1-x)$ and the sequences of heads and tails; the property mentioned here is that, at a coarse-grained level, they share the same statistical description: Prob (head)= Prob ($x > 1/2$). Noise is encapsulated in different ways into the dynamics according to the accompanying adjective: thermal, molecular (see below), white or coloured (that characterises its statistical features), additive or multiplicative. The noise is called 'additive' when the noise term is simply added to the evolution law as a forcing term, and 'multiplicative' when it affects the parameters of the evolution law. It has generally a more drastic impact on the solution in the latter case.

All investigations about the influence of noise on a state or behaviour, in particular robustness to noise, should first delineate the origin of noise and the setting in which it will be described.

(c) Statistical laws and macroscopic variables

A prominent example of a robust outcome arising from a stochastic process is provided by statistical laws assessing the occurrence of determined collective behaviours despite randomness and unpredictability of elementary events and individual behaviours. They have been fully investigated in probability theory, where each of N elementary events is described by a random variable X_i and their collective behaviour is associated either to the sum $\sum_{i=1}^{N} X_i$ or the product $\prod_{i=1}^{N} X_i$.

The most acknowledged is the law of large numbers, stating that $(1/N)\sum_{i=1}^{N} X_i$ tends to $\langle X \rangle$ when N tends to infinity (under some conditions of bounded variance and independence for the variables X_i). It can be illustrated in the game of heads and tails: each outcome is random, yielding equally a head or tail with no way to predict the result of a single flip, but the fraction of heads in a sequence of N independent flips tends deterministically to $1/2$ as N tends to infinity. Another statistical law, the central limit theorem, describes the fluctuations of the event empirical frequency around its limiting value in finite size $N < \infty$. It states that the relative fluctuations roughly behave as $1/\sqrt{N}$; more precisely, the difference between the empirical frequency and the probability, rescaled by $\sqrt{N}$ in order to go beyond the trivial convergence to 0 ensured by the law of large numbers, converges to a Gaussian distribution as $N \to \infty$. Both theorems show that accumulated fluctuations average out and large-scale collective behaviour gains a robustness that elementary events lack. Statistical laws show that it is precisely the stochastic nature of the elementary events that allows production of a robust deterministic outcome.

Turning from mathematics to the physical description of the overall behaviour of a many-body system (like a gas, spin lattice, or population), a key point is that most macroscopic variables are of sum type, namely defined as a sum of elementary state variables; current examples are pressure, temperature or local density. Central limit theorem states that they are essentially deterministic quantities with relative fluctuations scaling as $1/\sqrt{N}$ where $N \gg 1$ is the system size or number of elements [In some special situations, called critical points or critical phenomena, the correlations between the elements are long-ranged and central limit theorem does not apply (Castiglione *et al.*, 2008).]. Accordingly, a weakly correlated many-body system exhibits robust emergent features that are deterministic, reproducible and predictable insofar as the knowledge of individual statistical features thoroughly determines the observed features at the macroscopic scale.

Generalised theorems have been established to handle situations where the above-mentioned statistical laws fail, for instance when elementary random variables are long-range correlated or have an infinite variance. They delineate classes of random sequences, termed universality classes, each describing the range of validity of a different asymptotic law. The very existence of these

classes assesses the robustness of the corresponding asymptotic behaviours since they will withstand any perturbation that does not push the perturbed sequence away from its original class.

(2) Dynamic responses of noise

A different aspect of the relation between robustness and stochasticity is how a given phenomenon might robustly withstand or not the influence of external noise; this issue will be addressed by investigating the impact of noise on the underlying dynamics.

(a) Sensitivity to noise in critical situations

Any stochastic influence entering a non-linear system (as an input in an input/output device or as an additional forcing term in a dynamical system) experiences a non-trivial interplay with the non-linearities; this results from the simple fact that the average of a squared variable differs from the square of its average and, more generally, that non-linear relationships do not commute with statistical averaging. It is thus not obvious to foresee the average outcome of a noisy dynamics.

The current effect of noise is simply to shade the dynamics, to merge small-scale details into a seemingly featureless band, to smooth out the small variations into a band, and to turn marked branching points into fuzzy transition zones. But the influence of noise can be more determinant in special situations, termed critical, in which the macroscopic behaviour remains sensitive to microscopic details, for instance in the neighbourhood of a bifurcation and more generally in cases where the non-linear dynamics generates long-range correlations (in time or in space) or a continuum of possible solutions. In such situations, the noise will in general experience a drastic amplification of its strength and the outcome will be far more noisy than the input. But quite strikingly, noise can also emulate a coherent outcome, for instance it might trigger a transition, select one among several possible states or regimes, or turn the outcome into a bistable switch. Such a determined influence of noise has been evidenced on the velocity selection of a front propagating in a reactive medium (Lemarchand *et al.*, 1995), in noise-induced bifurcations, noise-induced order (Matsumoto & Tsuda, 1983) or in noise-activated processes and noise-enhanced barrier crossing preventing the system from remaining trapped in a metastable state (Horsthemke & Lefever, 1984). Noise can also induce the emergence of novel deterministic solutions, *e.g.* novel patterns in excitable media (Muratov, Vanden-Eijnden & Weinan, 2007).

(b) Stochastic resonance

A remarkable instance of a 'deterministic' impact of noise and noise-induced robustness of a deterministic dynamic response is encountered in stochastic resonance, namely a mechanism that occurs when a proper amount of noise is added to a weak deterministic periodic forcing. In physics, a resonance is said to occur when the response of a system to a deterministic periodic stimulus exhibits a steep maximum for a specific input frequency. It means that the system dynamics selectively enhances the impact of an input or driving force according to its frequency. In some non-linear systems, surrounding noise also might trigger a resonance phenomenon and enhance the impact of a weak deterministic and periodic driving force (Benzi, Sutera & Vulpiani, 1981). The paradigmatic example is a particle moving in a W-shaped bistable potential (a similar resonance can arise in threshold dynamics between the noise and a deterministic sub-threshold input) and excited with a weak deterministic signal of period T, too weak to trigger the transition between the two wells of the potential. When moreover some noise is present, one observes that the barrier can be crossed at determined optimal times (odd or even multiples of $T/2$ according to the crossing direction) provided the noise strength lies in a proper intermediate range. Indeed, the noise should be weak enough not to allow the transition alone but enough strong to provide the extra energy required to achieve barrier crossing at regularly spaced instants at which the deterministic signal induces a lowering of the energy barrier. Such lowering occurs once in each period and each crossing direction; the transition, fed by noise, is still a stochastic event hence some opportunities of barrier crossing might not lead to an actual transition and some periods be lacking; by contrast, the probability of an out-of-phase transition is negligible, hence there are no extra periods. In this way, the deterministic signal still imposes its periodicity onto the system behaviour. The synergy between the noise and the periodic signal is optimal when the characteristic time of the noise-induced transition (Kramers time $\tau_K \sim e^{\Delta U/\eta}$ where η is the characteristic energy of the

noise and ΔU is the energy barrier) equals half the period of the deterministic signal (Gammaitoni *et al.*, 1998). This mechanism of stochastic resonance has been identified in several situations, ranging from climatic changes like ice-ages where some noise enhances the slight oscillations of the terrestrial orbit orientation with periods of approximately 100 000 years (Ganopolski & Rahmstorf, 2002) to the functional role of sub-threshold signals in neurons, where internal noise helps them to trigger spikes (Hänggi, 2002).

The system behaviour is here robust insofar as the temporal pattern (oscillation) is shaped by the deterministic signal. Moreover, the robustness of the forcing influence of the deterministic signal and periodic system response is here increased by noise since it still observation of a periodic outcome even if the signal amplitude no longer reaches the excitation threshold.

(c) Numerical studies

A side issue about the dynamic response to noise of a system and its robustness properties concerns the relevance of its numerical investigation. Basically, since numerical noise due to round-off errors applies as a perturbation of the evolution law itself and hence has repercussions at all subsequent times, faithful numerical studies are inherently restricted to structurally stable properties. For instance, numerical investigation of a dynamical system automatically grasps the unique invariant measure (probability of presence in the phase space) that is robust with respect to perturbations while ignoring the non-generic ones, that will never arise in numerical results. Systems at a bifurcation point cannot be reached and bifurcations are evidenced only as fuzzy transition zones between two different well-defined regions; discontinuities, divergences and other singular features of the 'ideal transitions' are smoothed out. The width and fuzziness of these transition zones besides are directly related to the level of the noise perturbing the evolution law (Collet & Lesne, 1989). Numerical noise thus seems to restrict dramatically the use of numerical simulations in investigating the dynamics of a system. But quite counter-intuitively, chaotic properties somehow cure this limitation as far as statistical features only are concerned. Indeed, for a large class of chaotic deterministic systems ('hyperbolic' systems in technical terms), a remarkable theorem known as the shadowing lemma (Bowen, 1975) states that for any tolerance ϵ, there exists a maximal noise strength $\eta(\epsilon)$ such that any trajectory of the noisy dynamics remains close enough to an unperturbed trajectory, at a distance lower than ϵ, over the whole time. Although it is not possible to exhibit the actual trajectory from a given initial condition, it is enough to ensure that all the statistical features computed from the noisy trajectory provide good estimates of the statistical features of the actual dynamics. This theorem ensures that the statistical features of the dynamics can be reconstructed in a robust way from the observation of a noisy trajectory.

The general conclusion remains: numerical investigations give faithfully access only to the features of the system which are robust with respect to the addition of a limited amount of noise on both the variables and parameters of the system's evolution law or state equation.

(3) Stochasticity at work inside biological systems

We have presented general mechanisms accounting for the emergence of a robust outcome from stochastic events and in some cases, for the constructive role of noise. We shall now discuss whether they are relevant in living organisms. It will be argued that the emergence of spatio-temporal organisation (*e.g.* patterns, oriented motions, switches or oscillatory regimes) from underlying fluctuations is a hallmark of biological systems. It originates specifically in feedbacks tuning the fluctuations, and in selection and co-evolution of the statistical features of stochastic units and elementary processes, exerted by the overall structure and dynamics. Our questioning brings out that biological systems evolved so as to take advantage of internal stochasticity for ensuring both the robustness and the adaptability of their functional behaviour.

(a) Molecular noise, Brownian ratchets and molecular motors

Molecular noise is central in all intra-cellular processes due to inescapable Brownian motion of the molecules and inherent thermal stochasticity of molecular events like binding, chemical reactions or conformational transitions. Molecular noise should not be seen as a perturbation, since it is built-in: living systems have been obliged to manage with it since the very beginning. It has

been part of the evolution process and adaptation of the system, and belongs essentially to its reference setting and functional dynamics. The cell is a Brownian world, where inertia plays no role while fluctuations and stochastic events play a key role, all the more when low copy numbers of the considered species (*e.g.* DNA binding sites or transcription factors) prevent any statistical law from averaging them out. The upper limit for the size of a Brownian particle (that is, whose motion is a random walk in which acceleration plays no role) is reached when the buoyancy begins to overwhelm thermal energy ($k_BT/2$ per degree of freedom at temperature T, where k_B is the Boltzmann constant). This yields a maximal size of about one micron, called the colloidal limit. Below this limit, viscous forces proportional to velocities dominate the terms proportional to accelerations, and masses play no role in the dynamics whereas sizes and conformations matter. Chemical reactions deserve a stochastic treatment at sub-micronic scales: whereas mass action law is valid for describing test-tube experiments, it often fails inside the cells, either due to the small number of molecules involved (Barkai & Leibler, 2000) or to the crowded, diffusion-limiting and highly structured intracellular medium where reactions occur (Berry, 2002; Giavitto & Michel, 2003; Takahashi, Arjunan & Tomita, 2005). Derivation of mass action kinetics from a probabilistic description at the molecular level indeed relies on a mean-field approximation, in which fluctuations around the average concentrations are neglected and mean squares identified with square averages (Lesne, 2007). In consequence, one has *a priori* to consider a stochastic description of the intracellular dynamics and chemical reactions (Turner, Schnell & Burrage, 2004). Any discussion about the robustness of intracellular processes should account for molecular noise and tackle the issue of explaining how deterministic behaviours emerge at larger scales, *e.g.* expression profiles and cell types (Raser & O'Shea, 2005), determined cell cycles (Rao, Wolf & Arkin, 2002), or oriented motions. Let us detail this latter example.

At thermal equilibrium, the average displacement of a Brownian particle vanishes, even in an anisotropic medium. To get an oriented motion, a means would be to apply a field, but a biologically more feasible option is to harness the random motion by some filtering mechanism: the coupling to non-equilibrium fluctuations jointly with some anisotropy in the substrate can actually bias the random motion and endow it with a deterministic component and a finite average velocity (Jülicher, 2003). This is observed in Brownian ratchets and motor proteins moving on a filament with a periodic and asymmetric structure (Vandenbroeck, Meurs, and Kawai, 2005); they act as Maxwell demons able to sort motions and accept only the properly oriented ones, and feed on irreversible chemical reactions like ATP-hydrolysis to pay the free energy cost of this sorting. The functioning of a molecular motor can be seen as an instance of stochastic resonance (Section VI.2*b*) between the characteristic time of the out-of-equilibrium cycle through various conformations experienced by the motor protein, and the characteristic time of its thermal motion over one spatial period of the filament. As such, it inherits robustness with respect to transient perturbations of the protein shape or the chemical reactions, buffered within the sequence of stochastic events and inducing only a short pause, while the proper motion resumes once the perturbation has vanished.

(b) Internal 'mesoscopic' stochasticity

Only molecular degrees of freedom are inherently random insofar as they directly follow from thermal motion. Stochasticity at a supra-molecular level is of different nature. It is moreover relevant to distinguish extrinsic stochasticity, following from randomness in the inputs, initial conditions or boundary conditions (see Section VI.2), and intrinsic stochasticity, spontaneously resulting from a non-trivial collective effect. Indeed, investigating anomalous statistical laws (Section VI.1*c*) has shown that it requires very specific statistical features at the elementary level to escape the law of large numbers and central limit theorem: in order to maintain a stochastic behaviour at the level of an assembly, the elementary events should either have an infinite variance, exhibit strong correlations, the underlying medium should have a fractal-like structure, or the emergent features should exert feedbacks on the elements enhancing their variability. For instance, observing a random switch in a macromolecule conformation or random spiking activity in a neuron cannot be explained as a mere consequence of thermal fluctuations; it requires a specific regulatory architecture or dynamics (*e.g.* positive circuits or long-range correlations) channeling thermal fluctuations into a higher level stochasticity (Kholodenko, 2006).

Another mechanism invoked to account for apparent randomness in some biological processes

(neuron spiking, brain activity, heart beats) is chaotic dynamics and associated exponential amplification of initial errors and perturbations (Lesne, 2006*a*). In fact, it is not so different since chaos is a feature of a deterministic dynamics, that is, of an effective mesoscopic process also resulting from a non-trivial collective effect. Here again, the resulting large-scale stochasticity also drastically differs in nature from thermal noise. In any case, the non-trivial origin of mesocospic stochasticity hints at a selected functional role, that we shall now discuss.

(c) Adaptive role of stochasticity

In a biological context, internal stochasticity can be argued to promote adaptive answers in response to environmental variability. The proper adaptive effect of stochasticity is achieved by dynamic selection (elimination of unstable situations in the course of time) or natural selection (amplification of the fittest, see Section VII.1*b*). An example is the immune system, where variability is required in the 'assembly line' producing antibodies (it occurs at the genetic level, through recombination), so as to ensure the presence of a wealth of different antibodies potentially adapted to novel pathogenic entities, before these entities have been encountered; enhancement of the actually adapted antibodies then takes place according to the surroundings and its pathogenic content.

It has been suggested that certain mechanisms able to generate internal stochasticity have been selected and improved during the evolution of biological systems, in response to and to defend against surroundings variability (Pavé, 2007). It has been shown for instance that a strong increase in the mutation rate is triggered when a bacterial cell faces some stressful conditions (SOS system; Radman, 1975)). A recent '*in silico*' study also supports the natural selection of an optimal level of mutations between two successive generations (a joint selection of the mutation rate and genome length, balancing each other to maintain a constant number equal to roughly one mutation at each generation) (Knibbe *et al.*, 2007).

VII. ROBUSTNESS, ADAPTATION AND ADAPTABILITY

In a biological context, robustness should be confronted with two other properties of living systems: adaptation and adaptability. Adaptation ambiguously refers to both a process and its result; the process is the drift of the organism's features arising in response to some novel constraint or environmental change; the result is an organism adapted to its environment. Adaptability characterises its potentialities to do so. Both notions seem to be exactly the opposite of robustness, leading us to investigate how these three notions could be articulated. Several ways out of this paradox, all consistent with the lessons of the previous sections, will be proposed: first to take into account the time scale of the perturbations and their possible temporal structure (monotonous variation, oscillations, random drift), then to consider adaptation as a means of preserving or even improving the fitness, and finally to consider the very robustness of the adaptation and the selection of a proper level of adaptability, in an evolutionary perspective. By so doing, our analysis brings out nested levels of robustness, in parallel with nested levels of selection and correspondingly nested time scales. This view unifies the different instances of robustness that we have encountered in living systems in the previous sections, for instance functional robustness contrasting with robustness of the system state or constitution; it also enlightens in what respect there is a notion of robustness specific to living systems.

(1) Tension between robustness and adaptation

(a) Role of the time scale of the perturbation

The tension between robustness and adaptation is already present in any dynamical system. We argue that these two notions are in fact complementary since the response of the system to a perturbation essentially depends on the time scale of the perturbation compared to the characteristic times of its own dynamics. Consider for instance a perturbation $a \sin \omega t$ applied to an harmonic oscillator of bare frequency ω_0. Resonance occurs in the non-generic situation where $\omega \approx \omega_0$.

If $\omega \gg \omega_0$, the perturbation averages out over any interval $[t, t+\Delta t]$ such that $\omega_0 \Delta t \ll 1$ but $\omega \Delta t \gg 1$ (*i.e.* the time interval Δt is vanishingly small at the observable scale but it covers many periods of the perturbation), before the oscillator state has significantly evolved: the oscillator is thus robust to high-frequency additive perturbations. On the contrary, if $\omega \ll \omega_0$, the perturbation now varies with a slow time variable $\tau = \omega t$ and the oscillator regime is determined at constant τ, for each τ, hence at a fixed value of the perturbation, what is called the quasi-stationary or adiabatic approximation. The τ-dependence of the result is then restored, showing that the system follows the perturbation and exhibits a response adapted to its frequency ω. We see in this basic example how the same system can exhibit both a robust behaviour and an adaptive response to a perturbation, according to its frequency (respectively $\omega \gg \omega_0$ and $\omega \ll \omega_0$), as well as an hypersensitive response in some critical situations (here $\omega \approx \omega_0$), as discussed in Section VI.2*a*.

This conclusion applies more generally: robustness and adaptation of the same given feature are typically observed at different time scales. The adaptation process currently takes place on the long term, over several generations for an anatomical change (it then proceeds through natural selection) or at the time scale of the organism for physiological changes, like the immune response, the resetting of the circadian rhythm after a jet lag or the changes in blood composition following changes the surrounding oxygen concentration (it then proceeds *via* feedbacks or by natural selection at the cell level). Let us detail this latter example: in the case of a sudden decrease in oxygen concentration, the individual faints and then recovers if the decrease lasts only a very short time, otherwise it dies or recovers with strong after-effects, meaning that robustness with respect to this specific perturbation is very limited; in the case of a slight decrease in the external oxygen concentration, *e.g.* at high altitude, the organism adapts by producing extra erythrocytes to balance the reduction of oxygen concentration; - in the case of a slight but permanent decrease in oxygen concentration, the organism exhibits over the long term a slow adaptation at the species level towards a less oxygen-consuming metabolism.

(b) Dynamic selection versus *natural selection*

Selection of suitable changes in the organism state, metabolism, or behaviour is centrally at work in the process of adaptation; but it also has been invoked in the previous sections in relation to robustness properties. To clarify the respective role of selection in adaptation and robustness and to identify further clues on their distinction, we argue that selection takes place in two different modes. The first can be termed dynamic selection and relates to the viability of the system: systems that remain viable over a wider range of external conditions persist while others vanish and their matter or energy are possibly reused (think for instance of molecular complexes or sand dunes). It extends the notion of dynamic stability and expresses (somehow tautologically) that the more robust is a system state or behaviour with respect to surrounding stresses and constraints, the longer it will persist. This mode of selection is not restricted to living systems but applies to any entity submitted to degradation (or death) or arising as the stationary state of some dynamical system, *e.g.* assemblies, structures, or patterns. It is basically at work in all instances of self-organisation. We described in Section VI.2*a* the dynamic selection of a propagating reaction-diffusion front. A biological example is mitotic spindle formation and sister-chromatid separation: here the dynamic selection of the structure and motion emerging from several stochastic events is precisely what allows a functionally robust outcome to be achieved, namely equally separated chromosomes in the daughter cells, despite internal noise and variability in the ingredients.

The second instance, termed natural selection, originates in differential reproduction rates. Defining fitness as the efficiency with which heritable traits directly or indirectly impact on reproductive success, the population of the fittest lineage rapidly overwhelms the others. Any entity endowed with heritable multiplication abilities experiences such selection, for instance auto-catalytic chemicals, nucleic acids, cells or organisms; accordingly, whether it is a hallmark of life is still a matter of debate as fuzzy as the issue of delineating living and 'plain' physico-chemical systems.

Both instances of selection depend upon the time scale over which the different species or lineages are considered: a species might be successful in the short term but exhaust its source of nutrients and become extinct over longer periods (Rauch, Sayama & Bar-Yam, 2002). Accordingly, several fitness estimates, instantaneous or time-averaged over a tunable number of generations, are to be defined. It is currently stated that fitness never decreases, preserving acquired potentialities

and departing from them only for better ones [or a equally good ones in the neutral theory of evolution (Kimura, 1983)]. A species can nevertheless withstand a less adapted transient provided it is replaced with more adapted features before it becomes extinct. In consequence, the relevant fitness is a locally time-average one where such fitness-decreasing transients are smoothed out; at a given instant, we might observe non-equilibrium and sub-optimal features. Dynamic selection is responsible for 'active' elimination of misfit states, behaviours or individuals; natural selection acts in a different way, based on the fact that the fittest will multiply while the misfits will die with no or few progeny, and gradually become extinct. Nevertheless, this dynamic selection should not be too stringent, so that some variability in the relevant features of the individuals is preserved and differential-growth-based selection applies. Dynamic selection lies prior to natural selection since viability before and during reproduction period (or even after at the group level when the survival of older individuals helps younger ones to reproduce in better conditions) is obviously a prerequisite with a direct impact on fitness. But outcomes of dynamic selection are simply dynamically stable, whereas outcomes of natural selection are likely to be functionally robust (at least after enough evolutionary steps). The distinction between dynamic natural selection, acting at different time scales, parallels the distinction between physiological and evolutionary responses; it thus offers a clue to reconciling the robustness of physiological states and responses to signals with their adaptability over the long term.

We finally note that confronting robustness and adaptability of an organism is all the more intricate since robustness of features or behaviours during the organism's lifetime is a prerequisite for any selection and hence any adaptation to occur (Tautz, 1992). In the same spirit, genotype selection relies on the robustness of its relation to the phenotype, on which selection actually operates through its long-term reproductive success.

(c) Functional robustness and adaptation

Another link in the complex relationship between adaptation and robustness lies in the fact that the outcome of the adaptation process, having passed the double sieve of dynamic selection and natural selection in the new conditions, typically has a stronger functional robustness, meaning that the organism is able to cope (*i.e.* with no impact on its biological functions) with a continuously and randomly varying environment. A way to achieve functional robustness is indeed an adaptation of the dynamics. It is achieved by means of co-evolution of the underlying functional or regulatory networks (*e.g.* addition of nodes and rewiring, changes of the node characteristics, changes in the coupling strength along the edges) so that the proper regime is the globally stable one, spontaneously selected by dynamics for a wide range of initial conditions. Furthermore, the required changes of regime in response to stimuli or surroundings modifications should correspond to bifurcations of the dynamics triggered by these stimuli or modifications. It means that the networks have adapted in the course of evolution so as to exhibit the proper connections between a set of stimuli or surrounding conditions and a set of regimes, and now behave as multiple switches.

(2) Adaptive mechanisms

(a) Tension between adaptation and adaptability

A prerequisite for adaptation is the presence of several variants on which selection can act. Biological systems thus experience a tension between adaptation and adaptability (Ulanowicz, 2002), *i.e.* between the persistence of presently adapted structures and dynamics (*e.g* homeostasis, Section IV.3) and the possibility of variation to enable better fitting an ever-changing environment, the cost being the risk of becoming less fit and eliminated. We then encounter two options, already discussed in the context of cell differentiation (Section IV.7): either the suitable variation is triggered at the individual level by environmental change (instructive viewpoint) or some spontaneous variation is already present in the population and the environmental change only favours and enhances the fittest variants (selective viewpoint). The former 'Lamarckian' viewpoint is now disclaimed for organisms and in fact, it has become quite infamous [although curiously it is the ruling paradigm at the gene expression level, although some dynamic selection among clonal cells might occur and take advantage of their phenotypic variability, presumably originating in gene expression stochasticity (Raser & O'Shea, 2005), to favor the fittest cell phenotypes (Stockholm

et al., 2007)]. The latter 'Darwinian' viewpoint accounts for both adaptation and adaptability with no contradiction since being adapted and robust is a feature of individuals while becoming more adapted is a statistical feature at the population level, originating in the amplification of the fittest sub-population due to its relatively higher reproduction rate (Walsh, 2001). Some preexisting variability appears as the best means for a species to maintain its viability and fitness in a wealth of different and possibly not yet encountered conditions. It means that in living systems, robustness of structures and dynamics resulting from their adaptation should be balanced by an adequate level of variability ensuring their adaptability; such a compromise can be recognised at any stage and any level of organisation in living systems.

(b) Adaptive landscapes and canalisation

We have seen in Section V.2 that in several instances, the system evolution can be described as the motion of the point x describing the system state on the surface of a landscape $U(x)$, *e.g.* its free energy or its fitness. At the same time as Kramers' exploitation of the free-energy landscape paradigm to compute chemical reaction rates (Kramers, 1940), Waddington introduced the notion of epigenetic landscape as an image of environmental buffering during development, also encapsulating the robustness of a finite number of possible outcomes, the robustness of the developmental trajectory (that is, the choice of one among these outcomes) with respect to most environmental changes, and the sensitivity to specific ones at specific instants (Waddington, 1940). It also accommodates genetic buffering, that is robustness of the phenotype with respect to genetic variability. Genetic buffering has been argued to play a key role in evolution in allowing the accumulation of mutations with no impact in the phenotype until some mutation load threshold is crossed, leading to a markedly novel phenotype available for selection against the wild one (Rutherford, 2000). Waddington introduced the notion of canalisation to summarise in a single term the processes underlying these three features: finite number of possible outcomes, environmental buffering, and genetic buffering (Waddington , 1957; Kerszberg, 2004). Canalisation corresponds to the existence of compensatory feedbacks, reflected in a specially shaped relief, allowing the organism to reach the adult stage in a robust way, whatever its modulation in the course of development; it points to the necessity of considering gene networks instead of a linear regulatory path between a gene and its expression products, and at the role of redundancy [see Sections IV.8 and V.5 and Gibson & Günter (2000) for experimental evidence).

Enlarging the scope of the landscape paradigm, the notion of adaptive landscape is a way to account, through a continued modification of the landscape, for situations where the evolution or equilibrium rules are themselves evolving, due either to the direct influence of other systems (*e.g.* other species in case of a fitness landscape), or to the modification of the surroundings by the system of interest and others, mediating feedbacks and interactions respectively. Such extension also proved to be highly fruitful in evolutionary ecology; considering an adaptive fitness landscape for each species allows for instance investigation of the propensity of an intruder species to settle or not in a given ecosystem (Ferrière, Dieckmann & Couvet, 2004). In the context of epigenetic landscape, shaping the development and shaped by both genetic and non-genetic determinants (maternal factors, epigenetic markers, environmental factors), it amounts to considering that the very motion of the developing organism would modify the landscape on which it travels.

(c) Top-down mechanisms

Adaptive complex systems, in particular living systems, are characterised by a multi-scale organisation where an essential role is played by top-down mechanisms, also termed downward causation (Ellis, 2005); they originate in the ability of the superstructure to monitor local parameters, to modify the properties of the elements (*e.g.* a bistable behaviour replaces a single equilibrium structure) or to control their recruitment if an assembly process is involved. Such feedbacks from the macroscopic level and its emergent features onto the elements have a strong impact on the adaptive abilities of the system especially when they are able to induce bifurcation in the state, structure or dynamics of these elements. They propagate macroscopic constraints down to the microscopic structure and dynamics; accordingly, they are essential for adaptation in triggering a consistent drift of the system towards an optimised overall behaviour, at all its levels of organisation.

A biological example is encountered in gene expression, where the chromatin fibre structure

and dynamics might strongly affect the interactions between genomic DNA and transcription factors. For instance, cellular signals could induce appropriate conformational changes of the chromatin fibre and in turn locally control the binding affinity of DNA for specific transcription factors, according to an adapted scheme settled in the course of evolution (Lesne & Victor, 2006). Another paradigmatic example, in social science, is provided by cultural rules and laws generated by a society as a whole and having a strong impact on individual behaviour. These top-down mechanisms and their emergent nature have been claimed to make living (and artificial) systems irreducible to the physical and chemical laws ruling their ingredients (Polanyi, 1968; Ellis, 2005).

(3) Integrated multi-level view on robustness and adaptation

At this point, we are beginning to see a way out of the paradox addressed in this section VII: by considering robustness and adaptation at different time scales and different levels. We here propose a more systematic and clarifying view in terms of nested notions of robustness and adaptation.

(a) Individual and population levels

The distinction between dynamic and natural selections that we introduced in Section VII.1*b* recovers two acknowledged levels of explanation as regards robustness and adaptation: the individual level (where the individual is a cell or an organism) and the population level (Walsh, 2001). Functional robustness is a feature of an individual and its progeny. It can be considered as a generic property of the species if it is observed for almost all individuals. Contrary to robustness, adaptation is observed at the population level. It is a statistical phenomenon, corresponding to an evolution of the relative abundance of different lineages within the population. Typically, a new lineage generated by a mutation or environment-induced bifurcation, having a negligible weight in the population when it appears, overwhelms the others thanks to its fitness, that is, its better reproductive success; it becomes the typical lineage whose features are those observed at the population level.

The distinction between individual and population levels also matches that based on the time scales of the responses to perturbations and environmental changes (Section VII.1*a*). At the individual level, fitness evolves very slowly, through minute variations following from mutations and epigenetic changes. These variations are too slow to provide suitable adaptations at the individual level. But, by randomly accumulating over generations, they generate a wide diversity within a species, with more or less well represented variants. At the population level, the fitness is the weighted average of individual fitnesses, hence it could evolve faster simply by changing, over only a few generations, the relative representation of the different fitness variants and amplifying the preexisting subpopulation that is the most adapted to the new conditions.

(b) Nested notions of robustness

The analysis we conducted in the previous sections unraveled the relative character of the notion of robustness, that depends in particular on the kind of perturbations, as well as on the level and timescale at which it is to be observed. Also, the robustness of the elements is not straightforwardly related to that of their assembly or higher levels of organisation (on the contrary, robustness of the assembly typically requires some adaptability of its elements and plasticity of the interaction network, Section V.5). But the term robustness, especially in living systems, can refer to more deeply different notions, concerning features of different natures. We here argue that such a confusing diversity can be enlightened and its relation to adaptation and adaptability clarified by considering in a systematic way nested notions (notwithstanding for each one the variety of perturbations and time scales that could be relevant) namely robustness *(i)* of a state; *(ii)* of the mechanism leading to the observed stationary state; *(iii)* of a function (notwithstanding its mode of implementation in a given context, that has presumably to vary, precisely to ensure the robustness of the function) ; *(iv)* of regulatory mechanisms maintaining this function when the surroundings vary transiently; *(v)* of the adaptation of this function to better fit a permanent modification of the surroundings (Barkai & Leibler, 1997); *(vi)* of the evolution of the functional scheme following from successive adaptations; *(vii)* of the whole biosphere; it has been proposed that life robustness is that of a dissipative structure, offering a way to manage the energy input from the Sun (Hoelzer, Smith & Pepper, 2006).

Only the first two levels can be encountered in all systems including plain physico-chemical (inanimate) ones; the following levels, as for any statement involving functions and internal regulation, are restricted to living (and artificial) ones. Here appears the irreducible difference between physical robustness, with only two levels that can roughly be identified as dynamic stability and structural stability, and the hierarchy of robustness notions encountered in living (and artificial) systems. On mechanistic grounds, the existence of all these nested levels of robustness relies on the networked and multiscale regulatory architecture of the system. A still open issue is to determine how robustness 'crosses the levels'; in other words, what causal relations, if any, exist between robustness at different levels, how interlevel consistency settles, and conversely how inconsistencies and frustration turn to be a motor of evolution.

The nested picture we here propose embeds in a natural way the acknowledged ability of robustness itself to evolve and adapt its strength in living systems (Gibson, 2002). Our view amounts to considering not only the robustness of some structural or functional property, but also the robustness of the mechanisms ensuring this first-level robustness, and so on, along a more or less extended hierarchy according to the complexity of the system; each additional level in this 'robustness ladder' indeed requires additional control schemes hence a higher level of complexity.

On mathematical grounds, we can state formally the first levels of this hierarchy for an observable property X depending upon features Y (initial conditions, boundary conditions, parameters or inputs) in the case of a deterministic relationship of the form $F(X,Y)=0$. The robustness of the solution $X^*_F(Y)$ is determined by the value of the derivative $dX^*_F/dY = -F'_Y/F'_X$, the solution being all the more robust to perturbations δY when dX^*_F/dY is small. A second level of robustness is related to the impact of the variability of the rule F itself onto the solution; it requires to investigate the variations $X^*_{F+\delta F}(Y) - X^*_F(Y)$. Then, considering a one-parameter family $(F_a)_a$, a third level of robustness concerns the genericity of the succession of singularities encountered in the solution $X^*_{F_a}(Y)$ when a varies, namely whether the same succession is observed in different one-parameter families $(F_a)_a$, and under which conditions. In a dynamic context, where Y describes the initial condition and X the asymptotic state of the system in the phase space, these three levels correspond to *(i)* the dynamic stability of the attractor, *(ii)* the structural stability of the attractor and *(iii)* the genericity of its bifurcation scheme.

A noticeable point is that the failure of a lower-level robustness does not necessarily call into question robustness at higher levels. For instance, one issue is the robustness of a phase portrait, another is the presence in this phase portrait of separatrices indicating a lack of robustness of the asymptotic state with respect to initial conditions in the neighbourhood of this separatrix. Similarly, one currently investigates the robustness of a bifurcation scheme, where a bifurcation precisely marks a failure in the robustness of the asymptotic state with respect to variations in the control parameter.

(c) Hierarchical view on robustness, adaptation, optimality and selection

The above robustness hierarchy parallels the hierarchy of responses exhibited by the system after a perturbation or an environmental change. We might observe a modification of the state, that is, a response of the system; a modification of the dynamic parameters (*e.g.* kinetic rates, coupling constants, or transport coefficients) that is, an adaptation of the living system; a structural modification of the functional dynamics itself (*e.g.* some plasticity in the regulatory network and its dynamics) that is, an evolution of the living system. And so on, a higher level being for instance a change in the system evolvability. At each level, a qualitative change might follow from a perturbation of very low amplitude if the system is close to a threshold: a state close to the borderline of an attraction basin, a parameter close to a bifurcation value, or an organisation close to a viability boundary. The discussion in Section VII.1*a* extends to the whole hierarchy: the main determinant in triggering these different responses is the time scale of the perturbation or environmental change.

Reasoning on biological function and adaptation often invokes the so-called optimality of biological systems and pathways. But how could one be sure that evolution has already converged and achieved this optimality? We argue that the conundrum might be circumvented by considering nested levels of optimality, that parallels those introduced as regards robustness. Mainly, they correspond to fitness optimisation under constraints at different levels. They will be reached at

different time scales, and correspond to more and more stringent and specific adaptation. Taking as an illustration the case of metabolism, one might consider the optimality of some ingredient (among other existing ones in a given reaction), the kinetic rates in a set of coupled reactions, the reaction scheme required to produce some given molecular species, othe metabolic networks required to fulfill some function, or the whole multi-level organisation of the organism.

Not surprisingly, the same point applies to selection: beyond dynamic selection and natural selection discussed in Section VII.1*b*, selection of the sensitivity to evolutionary pressure and selection of the variability range, meaning selection of the evolvability of the species is at work in living systems, (Kirschner & Gerhart, 1998; Knibbe *et al.*, 2007). Evolution of the selection stringency and evolvability also are relevant at the level of ecosystems. We have underlined throughout this paper how closely the notions of robustness, adaptation, and evolution intermingle, appearing at the same time to impact and rely on one another. In order to disentangle their relationships, we propose to consider any living system as the ever-evolving result of nested selections: of the state, of the regulatory mechanisms ensuring the state robustness, of the mechanisms ensuring some variability in the regulation so as to allow adaptation of the state, of the time scale and range of this variability (its strength should increase and its time scale shorten in a stressful or highly variable environment). At each level, selection acts upon a different entity: individual for dynamic selection, population for plain natural selection, species for selection of the sensitivity to evolutionary pressure, ecosystems for selection of selection stringency. Its effect expresses itself in a statistical way, at the next level above, as an adaptation leading to a robust feature.

VIII. DISCUSSION

Back to our initial question: " Is there a notion of robustness specific to biological systems? ", we are now in position to conclude. The notion developed in physics and its correlates (stability, resilience, self-organisation) are certainly relevant in biology, but they are clearly insufficient to account for all aspects of biological robustness. As is well known, biological specificity lies in the fact that living systems are the result of an endless evolution following from natural selection. Through various examples, a more systematic mechanistic analysis and a novel integrative viewpoint, we here described how this (now quite obvious) difference between inanimate and living systems is reflected in their robustness properties and even in the relevant definition of robustness. More than in other fields, the use of the term 'robustness' alone is to be avoided in biology since its meaning and scope are strongly context-dependent.

The 'physical robustness' of the state of a living system, namely its persistence despite the presence of noise or perturbations, does not always coincide with functional robustness and fitness persistence, that define two alternative but essential notions of robustness pertinent to biological systems. We here argued that state persistence is the relevant feature with respect to short-lived or fast-varying perturbations, whereas it is adaptation to slower trends and even the robustness of adaptive abilities that matter at on evolutionary time scales. This point reflects the distinction to be made between dynamic selection and natural selection. Dynamic selection is sufficient for the establishment and persistence of the proper functional state after a stimulus or in given conditions (dynamic stability) but its functional robustness to all kinds of perturbation the biological system might encounter has been established thanks to natural selection on a far longer time scale. In fact, the very relation between the stimuli and the responses is a product of natural selection and ensuing evolution, that follows from the establishment and tuning of feedback loops and regulatory networks. Another striking manifestation of evolution is the way it has turned features currently conflicting with physical robustness, *e.g.* bifurcations or stochasticity, into an advantage promoting functional robustness. Within a regulatory scheme, a bifurcation acts as a switch; also, stabilisation of the system right at the bifurcation or transition point (which is possible in a regulated biological system) endows the system with an enhanced sensitivity to inputs or noise that could be beneficial, *e.g.* enlarging the signal range or generating the variability required for adaptability. As regards stochasticity, we have presented several instances in which a determined and robust outcome emerges from stochastic microscopic events: if self-averaging occurs and only the distribution is of

relevance at the level of the observed consequences, *e.g.* molecular collisions yielding the thermal motion of a Brownian particle, and yielding in turn the diffusion equation and Fick's law; if time correlations are strong enough so that a non-trivial (*i.e.* different from the statistical average) determined outcome emerges asymptotically (as in some neural assemblies behaving as a whole like a single effective neuron); if stochasticity is coupled to a periodic phenomenon as in stochastic resonance or molecular motors; if some regulatory mechanism is able to buffer the randomness (as in gene regulatory networks); if some selection mechanism is present that exploits the very randomness of the successive steps to adapt best to the surrounding conditions and inputs (as in immune system and recombination-based generation of antibodies). All these situations are encountered in biology and have been selected each in a different functional context. A remarkable conclusion is that, in several biological situations, the very variability of the elements ensures functional robustness of the collective behaviour; it means that the system itself is not robust as regards its structure and composition, precisely to offer flexible implementations able to accommodate changes in the constraints and external environment it has to cope with.

We have shown that the opposition between robustness and adaptation vanishes when one realises that the two notions actually stand at different levels and different scales. Furthermore, it also makes sense to consider the system in a wider scope and investigate the robustness of this adaptation. We argue that the relevant definition of robustness in a biological context is to consider nested notions: robustness of the state, of its regulation and homeostasis, of its adaptability. Some additional insights on robustness and adaptation can be found in Hammerstein & Hagen (2005), Hansen (2006), Krakauer (2005), Lewontin (1978), Kitano (2004) and Wagner (2007).

The multiple aspects of biological robustness and its expression leads to the notion of a robustness profile of an organism, delineating the set of external conditions and perturbations, impacting on various ingredients of the system at various scales, that would spoil or not the functions, over various durations. In a biological context, the robustness profile is relative to functions. It describes the domain of adequacy, in the set of possible [genotype, environment] pairs, between genotype or epigenotype (that is, epigenetic factors) and environment allowing the production of a functionally optimised phenotype. The boundary of the robustness profile (whose crossing corresponds to the appearance of dysfunctions and diseases) defines at the same time 'sensitivity to environmental factors' and 'genetic predisposition'; what is causal and induces the disease is neither the genome (and epigenome) nor the environment but their mutual ill-adaptation (Victor *et al.*, 2008)

IX. CONCLUSIONS

(1) The meaning of robustness is context-dependent: it makes sense only if we precise what is the feature whose persistence is under consideration and what is the set of perturbations a robust feature will by definition withstand.

(2) Robutsness of physical features stems from various (non exclusive) mechanisms: conservation laws, symmetries, variational principles, dynamic stability, structural stability, that are also relevant in a biological context.

(3) Robustness of a biological system embeds but cannot be reduced to the notion developed in physics: mainly, it is essential to distinguish structural robustness and functional robustness. The latter is specific to biological system and it is achieved thanks to regulatory mechanisms, *e.g.* feedback loops.

(4) The specifity of biological robustness originates in the fact that living systems are the product of evolution and continuously experience natural selection, whereas physical systems (including biological systems as a peculiar subset) experience only dynamic selection of stable states.

(5) Stochasticity is often, but misleadingly, considered as an obstacle to robustness. Statistical laws for instance show that a robust determined outcome can follw from stochastic elements. Furthermore, in biological systems, stochasticity in the elementary processes provides flexibility promoting functional robustness in a variable environment; it is also essential in generating diversity on which natural selection could apply and lead to adaptation.

(6) Nested notions of robustness, relevant to different time scales and different levels of organisation, allow one to reconcile the seemingly contradictory requirements for robustness and

adaptability in living systems.

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