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Multiscale analysis of biological systems

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Abstract It is argued that multiscale approaches are necessary for an explanatory modeling of biological systems. A first step, besides common to the multiscale modeling of physical and living systems, is a bottom-up integration based on the notions of effective parameters and minimal models. Top-down effects can be accounted for in terms of effective constraints and inputs. Biological systems are essentially characterized by an entanglement of bottom-up and top-down influences following from their evolutionary history. A self-consistent multiscale scheme is proposed to capture the ensuing circular causality. Its differences with standard mean-field self-consistent equations and slow-fast decompositions are discussed. As such, this scheme offers a way to unravel the multilevel architecture of living systems and their regulation. Two examples, genome functions and biofilms, are detailed.

Keywords Multiscale approaches · Effective models · Regulation · Circula causality · Self-consistent equations · Integrative Biology · Systems Biology

1 Introduction

Biological functions involve processes at various scales. This statement is obviously true for organismic processes like development, or for a bacterial colony. It is already relevant for intracellular functions like gene expression and signaling pathways. Understanding biological functions requires to *integrate knowledge or data of various natures*, available at various levels, and described *within*

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various frameworks, from quantum mechanics (for elementary processes like light transduction) to stochastic kinetics to deterministic rate equations and continuous medium theory (e.g. elasticity theory). Beyond the epistemic issue of capturing a real process in descriptions and measurements prescribed by our own abilities and scale limitations, biological systems present a greater challenge: they are *intrinsically and irreducibly multiscale processes*. Regulation of a biological function has to bridge the state of the cells and some surrounding features with basic mechanisms at the atomic or molecular scale, in an adaptive way. A cell itself has to perform a multiscale integration. For instance, transcription in eukaryotes relies at the same time on information about DNA sequence and bound proteins, histone chemical status (e.g. acetylated or not), chromatin conformation (e.g. condensed and topologically constrained or not), nuclear localization (e.g. near a nuclear pore), cell state and its surroundings; it involves influences from each of these various levels, either directly controlling the polymerase binding and activity, or being mediated by signaling pathways or mechanical constraints. Our analysis and modeling should follow the same line (Lesne et al. 2012, Lesne and Victor 2006). A similar challenge is met in (Karsenti et al. 2007) in the context of microtubules assemblies and in (Muskhelishvili et al. 2010) in the context of bacterial transcriptional regulation. I will argue that the multiscale organization of biological systems has been established by natural selection and ensuing coadaptation of their various parts and levels. Specific multiscale approaches are thus to be devised for their integrated understanding. A possible one, based on a self-consistent procedure, is proposed in this paper, Sec. 9.

2 The example of genome functions

In biological systems, one aims at understanding *functions*, whose fulfillment in general involve specific and adapted features at several scales. Let us consider the example of a genomic process, e.g. replication, transcription or repair. Its initiation and regulation typically involve specific DNA sequences (e.g. TATA box or some other specific binding site), nucleotide relative positioning (e.g. in DNA restriction by a ribozyme), specific nucleosome conformations with spatial alignment of histone residues (e.g. nucleosome gaping (Mozziconacci and Victor 2003) or reversome conformation (Bécavin et al. 2010)), nucleosome positioning, post-translational modifications of histone tails (Jenuwein and Allis 2001), proper conformation of the chromatin fiber and even fiber positioning within the nucleus (Spector 2003). A relevant explanatory model has to somehow keep track at the same time of the chromatin loop location and conformation, nucleosome shape, histone tails chemical status and certain atomic details. This requires to articulate investigations at several scales (Lesne et al. 2012, Lesne and Victor 2006) for instance:

(i) all-atoms simulation to determine base-pair or residue precise positioning,

(ii) molecular mechanics, Brownian dynamics or inverse kinematics to determine the position of linkers or histone tails and account for the presence of the linker histone H1 and histone-tails post-translational modifications,
(iii) mechanical modeling of the 30-nm fiber and topological features of the chromatin loop (i.e. topologically closed stretch of chromatin fiber delineated by boundaries or insulators) at the scale of about 100 kbps.

The challenge is here to take into account the *reciprocal influences* between the levels, e.g. to describe residue positioning *within the chromatin fiber*.

3 The example of biofilms

Biofilms are complex structures formed by a (often multispecies) bacterial colony and a secreted matrix. One of their major functional features is their organization at several scales, from that of a single cell up to macroscopic scales. To understand the growth of a biofilm, its structural properties (density, porosity, thickness), its mechanical properties (attachment/detachment under the action of a flow), or its activity in consuming substrate (e.g. in applications to waste treatments), we have to describe jointly the individual and the global level. Microscopic simulation can be developed to give an explicit basis to effective macroscopic models of biofilms and provide a framework to integrate experimental data (Deygout et al. 2011). Two main types of microscopic simulation are usually considered: *cellular automata*, describing the evolution of the particle contents of microscopic spatial cells according to simple rules involving only the states of the neighboring cells, and *individual-based models*, also called *agent-based models*, prescribing the behavioral rules of each individual particle. Both can be used to integrate microscopic knowledge and e.g. determine the explicit expression of local rate constants, to be plugged in macroscopic models in terms of ordinary or partial differential equations. Such a bottom-up approach is well-suited to take into account refined microscopic mechanisms or complicated geometry at the bacteria level.

The challenge is here to simultaneously take into account top-down influences, for instance to describe the physiological behavior of a bacterial cell *within the biofilm*, with e.g. possible functional changes according to its local surroundings and spatial location.

4 Bottom-up approaches using effective parameters

A first way for implementing a multiscale analysis is to travel across the scales in the bottom-up direction. This way is the standard one in physics and it has produced both technical contributions (for instance the whole domain of statistical physics, see (Castiglione et al. 2008)) and epistemological insights (see for instance (Anderson 1972; Simon 1962)). A basic notion is that of *effective parameter*, encapsulating the net result of several (complicated or not fully known) mechanisms into a single quantity parameterizing a structural feature,

an interaction, or a contribution to an evolution law at a higher description level. Bottom-up approaches aim at determining the collective behavior of an assembly of elements. Effective parameters play an essential role in this integration, in reducing a wealth of complicated and possibly not fully known microscopic ingredients to a single effective one, *having the same impact at higher scales*. Henceforth, a tractable bottom-up integration will typically be hierarchical, with the first steps devoted to the design of a *minimal model* for the elements, and the next steps to the determination of the emergent features of the assembly. Let us cite a few examples of effective parameters and associated dimensional reduction.

• *Rate constants* involved in chemical kinetics are derived under simplifying assumptions from the stochastic (possibly quantum) analysis of the reaction process. Within Kramers theory, they are expressed as a function of the temperature and the limiting-step free-energy barrier (Hänggi et al. 1990). The concept of reaction rates depending on the temperature has been introduced on empirical grounds by Arrhenius in 1889, about fifty years earlier than Kramers.

• Embedding microscopic fluctuations into a macroscopic dynamic model can be done by adding an *effective noise term* to deterministic ordinary or partial differential equations describing the dynamics at large scale. These stochastic differential equations are called *Langevin equations*, by extension of the equation introduced by Langevin (1908) to account for Brownian motion. This noise term appears as the net result of microscopic degrees of freedom that we do not intend to take into account (Lemarchand et al. 1995). This amounts to cutting off high frequencies and short wavelengths and replacing the detailed description of the most microscopic modes by an effective noise term. Stochastic calculus has been developed to handle such equations (Gardiner 1983), however its efficiency is limited to special kinds of noise terms (white or colored noises). Another way is provided by *stochastic processes*, whose evolution rules are essentially random, for instance a Markov chain whose dynamics is fully prescribed by the *probabilities of transition* between the instantaneous state and the following one. These probabilities appear as the effective parameters of the model. In contrast to Langevin equations, Markov processes can cope with discontinuous forms of noise (Van Kampen 1981).

• An *effective diffusion coefficient* D_{eff} can account in an average way of microscopic heterogeneities that are present within a porous substrate provided they have a finite characteristic size a. At mesoscopic scales $dx \gg a$, diffusion is described using a a plain diffusion equation, with a spatially constant diffusion coefficient D_{eff} and simple boundary conditions at the border of the sample (Lesne 2006; Nicholson 2001). Computing an effective diffusion coefficient is an instance of a general method called *homogenization* (Hornung 1997; Torquato 2002), intensively developed in the context of composite materials (Mathias et al. 2006). The intuitive justification has been supplemented with a mathematical analysis to determine what is the appropriate "representative volume", that is, the size dx of the regions considered as the elementary volumes of the homogenized system (Ben Arous and Owhadi 2003) and in which

all the microscopic structures and processes will be averaged: dx has to be large compared to the characteristic lengths of these structures and processes.

• The *relative dielectric constant* $\epsilon_r \approx 80$ accounts for the electrostatic influence of water by replacing ϵ_0 by $\epsilon_0\epsilon_r$ in electrostatic interactions and Maxwell equations. This effective description, termed "implicit solvent" is a good approximation at supra-molecular scales. It fails when only a few hydration shells are involved, as in computation of RNA tertiary structure or protein-protein interactions. An explicit description of water molecules is then required.

• In some instances a nucleosome can be described as a *solid body* (Ben Haïm et al. 2001). Two parameters are sufficient to reproduce the main structural and elastic features of the 30-nm chromatin fiber: the angle α between the DNA left-handed helical path onto the histone core and the core axis, and the *angle Φ between the incoming and outgoing linkers*. This angle Φ is a typical example of an effective parameter: it is considered as a tunable quantity in the study of the fiber structural and elastic properties (Ben Haïm et al. 2001; Woodcock et al. 1993) without entering the details of the molecular determinants responsible of its value and variations. It would be another issue to relate the precise description of a nucleosome and its surroundings (ionic strength, presence of histone H1, presence of polyamines, post-translational modifications of histones, non-histone binding proteins) with the value of Φ.

• In more refined functional studies (Bancaud et al. 2007; Mozziconacci et al. 2006; Sivolob et al. 2003; Wong et al. 2007), nucleosome conformational transitions have to be taken into account. It is often enough to consider possible switches between a finite number of conformations, i.e. discrete states, (Bécavin et al. 2010). and describing the nucleosome as an effective shape characterized by a few effective parameters remain valid.

• DNA entropic elasticity can be described using an effective continuous model, the *worm-like-chain* model (Kratky and Porod 1943). DNA molecule, although composed of discrete atoms, is identified above nanometer scale with an homogeneous one-dimensional semi-flexible filament and entirely described by its local curvature $\rho(x)$ where x is the arc length. This model involves a single effective parameter, the *persistence length* $\mathcal{L}_p$, defined by the expression of the energy density $\mathcal{E}(x) = kT\mathcal{L}_p\,\rho(x)^2\,/2$ at the temperature T. This description does not intend to take into account sequence effects, that require a description at a finer scale or an effective description in terms of disorder.

As shown by the above examples, an effective parameter can relate a digital (i.e. discrete) description with an analog (i.e. continuous) one, for instance encapsulate the entropic elasticity of a discrete sequence of DNA base pairs in the persistence length of a continuous elastic filament, or conversely describe the wells of a continuous energy landscape as discrete states (Lesne 2007). Most often an effective parameter should have a specific use: it makes sense only in the dimensionally-reduced description in which it is involved, and with regards to the integrative study in which it is involved. Considering e.g. a solid shape for the nucleosome is relevant on geometric and topological grounds but

not necessarily for dynamic studies. An effective parameter does not necessarily share all the interpretations and properties satisfied by the corresponding bare parameter, if any, despite sharing the same name: An effective diffusion coefficient does not necessarily satisfy the Einstein relation linking diffusion and viscosity. An experimental force-extension curve of a chromatin fiber can be fitted within a worm-like-chain model, considering the fiber as a continuous filament with a single elastic degree of freedom, bending. The fit yields an effective persistence length $\mathcal{L}_{p,eff}$ (Bystricky et al. 2004). But this length $\mathcal{L}_{p,eff}$ should not be confused with the actual bending persistence length $\mathcal{L}_p$ of the fiber when it is described within an elastic-worm-like-rod model (Ben Haïm et al. 2001), that is, as an elastic rod with three elastic degrees of freedom (bending, twisting, stretching). Effective parameters will play a central role in the self-consistent integrative approach proposed in this paper (Sec. 9), as the loci where some top-down control can be specified

5 Top-down approaches using effective constraints and inputs

Another way of analyzing a multiscale system is to investigate *top-down* relationships, namely how macroscopic inputs, structures and constraints might affect the features of the constitutive elements and the elementary processes. Such influences are often termed *top-down causation* (Ellis 2005). Again effective quantities are useful to encapsulate in a low-dimensional expression (e.g. a field, a force, a geometrical constraint, an energy landscape, a source term or a boundary condition) involving only a few parameters, a wealth of top-down influences. When external constraints apply via boundary conditions, it may be useful to process these conditions into local prescriptions at work inside the system. For instance, an external concentration (e.g. of oxygen) may be replaced after suitable computations involving assumptions on the diffusivity and decoupling between diffusion and consumption, by an effective distribution inside the system. Effective noise terms can be introduced to account for a variety of ill-identified external influences, for instance an high-dimensional input that we do not want to describe in detail. What matters is only the resulting influence on the system at the chosen level of description.

A benefit of effective descriptions, either reducing the description of the elements (effective parameters) or the description of the surroundings (effective inputs and constraints), is their *parcimony*. Because neither the microscopic details nor the macroscopic surroundings are fully known, often not even all identified nor fixed, the models should involve only a coarse description, so as to avoid over-interpretation or spurious sensitivity of the results to the precise knowledge of what is taken for granted in devising the model. This requirement of parsimony does not intend to mean that the biological reality is bound to be that simple, but that our description has to be *unbiased* and *robust* with respect to an additional detail. This is all the more demanded since the intrinsic variability observed between identical biological systems, e.g. cells within a clonal population, superimposes to the variability of our observations

and fuzziness of our knowledge. Another option would be to explicitly take into account this intrinsic variability in the modeling, which would require a (more complicated) probabilistic setting.

6 A basic classification: plain, critical and complex systems

At this point, an essential distinction in the multiscale logic of physical and biological systems has to be underlined.

- In plain (mostly physical) systems, microscopic fluctuations average out. Macroscopic observables can be identified with average quantities and described by deterministic continuous fields, that obey ordinary, partial or integro-differential equations with no mention of an underlying microscopic level. Standard examples are classical mechanics, hydrodynamics (Navier-Stokes equations) or chemical kinetics (mass action law).
- In striking but less frequently encountered *critical phenomena*, fluctuations are enhanced by long-range correlations and persist at all scales up to macroscopic ones. Microscopic fluctuations are thus able qualitatively modify macroscopic behaviors, typically leading to anomalous laws.
- In biological systems, situation is yet different. Microscopic fluctuations are either *buffered by regulatory circuits*, either exploited and possibly amplified as a *source of variability* feeding selection-driven adaptation mechanisms. In the latter case, microscopic fluctuations potentially have repercussions at all scales. In the former case, they are controlled from above by means of feedback circuits that monitor the microscopic ingredients so as to get the proper macroscopic regime and ensure its maintenance.

This tripartite categorization has a parallel formulation in terms of the relevant number of degrees of freedom. In plain physical systems, *a few collective variables*, defined as averages over the microscopic degrees of freedom, are sufficient to describe the macroscopic behavior. Both critical systems and biological systems depart from this case by exhibiting *very many coupled degrees of freedom*. Averaging is no longer efficient to bring out the essential behavior. In case of critical systems, the efficient strategy is a recursive integration and associated renormalization-group methods (Castiglione et al. 2008, Lesne 1998). In biological systems, I claim that dimensional reduction should be achieved jointly at all scales in a *self-consistent way*, replacing at each level several degrees of freedom by a few effective terms reciprocally coupled (Sec. 9).

Biological systems are a specially wide and important instance of *complex systems*, which can be generally defined as assemblies of interacting elements where emergent features directly or indirectly *modify* the elements. Such a logical scheme is often termed *circular causality* (Muskhelishvili et al. 2010). This term underlines the coupling of bottom-up and top-down relationships, leading to self-organized and possibly adaptive behaviors. Typically, elements collectively modify their surroundings, in a way sufficient to influence back

the elementary interactions, which in turn may change the collective behavior of the assembly. Only a few purely physical systems display such features, for instance sand dunes (Hersen et al. 2004) or coast reliefs (Werner 1999). In the case of sand dunes, the complexity originates in a change in the interaction between the wind and the sand heap when the heap has passed some critical size; the heap then behaves as a whole, a "dune", able to modify the wind in its vicinity. It is then qualitatively different from a small heap of grains which only experience the influence of the wind without exerting any feedback on it.

7 Mean-field self-consistent approaches

My proposal is that a good strategy to solve the chick-and-egg problem raised by the circular causality of living systems is to bridge in a self-consistent way the bottom-up and top-down effects. This does not sounds new: in statistical physics, self-consistent equations are encountered for a long time in *mean-field approaches*, in which the influence onto a given microscopic elements of all the other ones is expressed as a function of some average quantity. However, the circular causality apparent in their formulation is far different from the stronger instance observed in living systems. Let us substantiate this claim by discussing the historical example of mean-field study of ferromagnetism.

A ferromagnetic system is described as an assembly of N spins located at the nodes i of a spatial lattice, each experiencing pairwise alignment interaction with its nearest neighbors and possibly the influence of an external magnetic field h_0. Our macroscopic perception of this system of interacting spins reduces to the overall magnetization M. In the standard mean-field approximation, correlations between the spins and spatial heterogeneities are ignored. Accordingly, macroscopic observables describing the collective behavior can be identified with statistical averages of the microscopic features according to the law of large numbers, that is, $M \approx N\langle s\rangle$. The trick is to replace the resultant influence on a given spin s_i of all its neighbors by that of a *mean field* $h_{eff}(M) = aM$ where a is some multiplicative factor depending on the interaction strength. Henceforth spins can be formally considered as independent entities, whose average magnetization M is given by a well-known formula of the form $M = B(h)$, see e.g. the textbook (Laguës and Lesne 2011). Plugging in the field expression $h = h_0 + h_{eff}(M)$ yields a self-consistent equation $M = B(h_0 + aM)$. In considering that the same uniform and deterministic field $h_{eff}(M)$ applies identically onto each element, local fluctuations and correlations between the elements are neglected. Accordingly, the validity of mean-field approaches fails in case of long-range correlations generating fluctuations at all scales. Such critical situations require renormalization-group approaches (Castiglione et al. 2008; Lesne 1998).

This example introduced a general method to determine the macroscopic behavior of an assembly of interacting elements, termed a *mean-field approach*. Formally, the behavior of the system can be properly formulated via *self-consistent equations*, expressing the identity of the macroscopically observed

quantity M, and the collective quantity $\mathcal{M}_N[s_1(M), \ldots, s_N(M)]$ resulting from the interaction of N elements with state s_i. The dependence $s_i(M)$ of the individual states s_i on M is an *approximation* of the resultant influence on a given element i of all the other ones. I underline that *only interactions between the elements are at work* in such systems. The expression $s_i(M)$ provides a closure relation yielding a self-consistent equation involving only the macroscopic variable M: $M = \mathcal{M}_N[s_1(M), \ldots, s_N(M)]$. It has the mathematical expression of a fixed-point equation: $M = \Phi(M)$. In practice, when a direct analytical resolution is too tricky, the solution can be obtained recursively, given an initial value M_0 and computing $M_{n+1} = \Phi(M_n)$, provided the recursive scheme converges; the limit is the desired value M.

8 Slow-fast decomposition

The term "multiscale analysis" evokes a more specific method encountered for long in dynamical systems theory. This method is devised to solve a special kind of coupled evolution equations where a small parameter $\epsilon \ll 1$ enforces a time-scale separation between slow and fast components in the dynamics. The common physical wisdom identifies slow variables to macroscopic observables and fast variables to microscopic degrees of freedom, however this intuitive interpretation is not necessarily true for living systems.

The typical instance is an ordinary differential equation where a multiplicative small parameter appears in front of a highest-order time derivative:

$$\begin{cases} \epsilon dx/dt = f(x, Y) \\ dY/dt = g(x, Y) \end{cases} \tag{1}$$

The dependence on ϵ is termed *singular*, insofar as the behavior of the solution for $\epsilon \to 0$ qualitatively differs from the behavior of the solution for $\epsilon = 0$. Equations of this kind are known for long in control theory (the slow variable Y is controlled by the experimenter and the slaving of the fast variables x to Y is designed by the engineer) and in physics (where the slow variable Y is some macroscopic observable and the dependence of the fast variables x onto Y is usually derived using mean-field-like arguments). The idea is to exploit time-scale separation to decouple slow and fast variables and get formally independent time evolutions. The first step is to solve the equation for the fast variable x at fixed value of Y. This step, called a *quasi-static* or *parametric* approximation since Y is considered as a constant parameter, yields the value $X(Y)$ such that $f[X(Y), Y] \equiv 0$. Then, focusing on the slow behavior, we plug the stationary value $X(Y)$ into the evolution of Y to obtain a closed equation:

$$dY/dt = g[X(Y), Y] \equiv G(Y) \tag{2}$$

This second step is called a *quasi-stationary* approximation since it amounts to identify the fast variables with their stationary value (i.e. such that the right hand-side f of dx/dt vanishes) in the slow evolution. The small parameter ϵ

re-enters when, in a possible third step, one looks for the detailed evolution of the fast variable x (Lesne 2006, Nayfeh 1973). Fast variables elimination when the fast dynamics is non-autonomous can be found in (Artstein 1999).

Based on scale separation, slow-fast decoupling methods may be seen as a temporal analog of a mean-field approach (Lesne 2006). Plugging the closure relation $x = X(Y)$ into the dynamics of Y yields a dimensionally reduced self-consistent equation for the macroscopic variable Y. These methods illustrate the general philosophy according which reduction of the dimension of the complete description and extraction of a relevant macroscopic description may be achieved in describing only quantities varying only at macroscopic scales (Castiglione et al. 2008; Givon et al. 2004). In a biological context, this approach has been implemented e.g. in the study of biochemical networks (Radulescu et al. 2008). Often a clue is to consider the evolution of quantities averaged in phase space, for instance the moments of the microscopic variables (Dieckmann and Law 2000) or aggregated variables associated with a coarse-grained version of the initial model (Auger et al. 2008; Gaveau et al. 1999). Time-scale separation is also a strong argument to eliminate fast oscillating variables X by averaging their oscillations at fixed Y and plugging their average value $\langle X \rangle_Y$ in the equation describing the evolution of the slow variable Y (Bogoliubov and Mitropolskii 1961; Nayfeh 1973; Sanders et al. 2007). Derivation of the dimensionally-reduced dynamics for time-averaged variables is described e.g. in (Acharya and Sawant 2006).

In the above analysis, self-consistent equations are given. In practice, it is another matter to get there and to delineate slow and fast variables. I claim that a radically new approach is necessary to fully capture the multiscale logic of living systems. Indeed, due to its evolutionary imprint, the articulation between the levels of organization of a biological *system* cannot in general be expressed as a system of coupled equations for the local and the global variables. Each level often requires to be described in its own formalism, thus reducing the applicability of standard slow-fast decomposition methods. Singular perturbations and decoupling of slow and fast variables remain relevant in a biological context in cases where a local mechanism or functional process can actually be described using a coupled set of *differential equations* involving a small parameter in the above singular way. A well-known example is the derivation of Michaelis-Menten kinetics for enzymatic catalysis; the small parameter ϵ is there related to the ratio of enzyme concentration to substrate concentration (the enzyme is present in minute amount), and the fast variable is the concentration of the intermediary complex made of the enzyme and the substrate (Lesne 2006). Other applications can be found e.g. in the context of population dynamics (El Hajji and Rapaport 2009).

9 Unravelling the coevolved consistency of living systems

Considering now living *systems*, the relationship between the state or evolution of the elements and the higher-level processes or superstructures cannot,

most often, be expressed as a system of coupled equations for the individual and collective variables using mean-field-like arguments. Natural selection has been at work, at the organism and even at the species level, to produce the observed functioning of living systems. The ensuing coevolution of the various components of organisms led to the appearance of tinkered entities (like allosteric enzymes) and coordinated processes, which cannot be understood using only physical arguments. I mean here that their appearance do not correspond to any reasonably probable physical event. This evolutionary design of living systems biased the generic physical behavior and harnessed the physical laws to produce adapted and regulated organisms. Although never being in contradiction with the laws of physics (this question was warmly debated in the past), living systems differ from inanimate systems in an irreducible way (Polanyi 1968, Schrödinger 1944).

To substantiate these claims, let us go back to the example of ferromagnetism (see Sec. 7). A spin does not "know" that it participates to the mean-field h_{eff}; it only experiences interactions with its nearest neighbors. In contrast, although the overall conformation of a chromatin loop is determined by the assembly of nucleosomes and DNA, this loop actually behaves as an autonomous entity, endowed with a topological invariant, its linking number. Due to this conservation law, any local DNA or nucleosome modification will be perceived at any other place. Another example is transcriptional regulation in a bacterial cell (Muskhelishvili et al. 2010). The cell is sensitive at the level of its metabolism to a change in the environment. It experiences a metabolic switch which in turn controls an adapted switch in gene expression via a coordinated interplay between the pattern of DNA-bound proteins, the molecular composition of the polymerase and the spatial distribution of DNA supercoiling. The association of these factors is an instance of coadaptation achieved by means of natural selection, with no physical necessity In living systems, the evolutionary imprint reflects in a stronger downward causation than in physical systems. As if, in ferromagnetism, some source of variability would have produced different values for the spins and natural selection would have selected the distribution of values yielding the best magnet (best in a sense to be specified according to the biological context, either the most efficient, or the most robust, or the most adapted, or the most tunable).

My point is that self-consistent approaches can be extended far beyond mean-field models and tailored to capture the above specificity of living systems. This point has been addressed several times on epistemological grounds, e.g. (Ellis 2005, Polanyi 1968) but more rarely as a practical methodology. Let us explain the scheme in general terms before giving some examples. Effective parameters involved in the minimal modeling of elementary actors (Sec. 4) are seen as the loci where some functional knowledge about top-down regulation can be injected: dependence of effective structural features (e.g. the gaping angle) of the nucleosome onto the overall conformation of the chromatin fiber and higher levels of organization, dependence of the effective parameters describing the nucleosome structure on the level of supercoiling of the chromatin fiber, dependence of the effective bacterial growth rate onto some features (assumed

or measured) of the biofilm. In a complementary step, some prior analysis or experimental knowledge is used to delineate how effective constraints or inputs encapsulating top-down influences in a compact way depend on the elementary features and mechanisms. In short, each level embeds entries that are fed by the other levels, and produces outputs feeding the other levels, as represented in Fig. 1. Running the different parts of the model in following this loop should yield a stable picture, in which inputs and outputs of each part are no longer updated. This achieves the integration of bottom-up and top-down influences and yields an "operationally closed" description, according to the terminology used in (Muskhelishvili et al. 2010).

In the case of biological systems, in contrast to self-consistent approaches encountered in physics, the relations $h = H(r)$, Fig. 1, expressing how the features h of emergent processes and superstructures depend on the effective parameters r describing the elements, do not simply follow from an averaging approach. Neither do the relations $r = R(h)$, capturing how the elements r are affected by the higher-level features h, follow from a mean-field approximation. They involve a wealth of intermediary mechanisms and ingredients, all devised and tuned by numerous runs of natural selection. In particular, these two relations are not simply the inverse one of the other (in which case the self-consistent scheme would be a mere tautology). Part of the articulation may be done"by hand" using some biological knowledge, mainly the identification of the coordinating go-between (Bécavin et al. 2010, Lesne et al. 2012, Malo et al. 2010, Malo et al. 2012) allowing us to bridge in a mechanistic way what is otherwise a statistical relationship (i.e. a correlation). This bridge, termed *structural coupling* in (Muskhelishvili et al. 2010), amounts to a shortcut of the (often contingent) evolutionary history. I underline that the aim is to devise self-consistent *scheme*, not necessarily within the restricted setting of self-consistent equations. Each level may demand to be described in its own relevant formalism or theoretical framework. Accordingly, the microscopic variables r and the macroscopic variables h may well belong to different settings, e.g.the variables r to a molecular dynamics or multi-agent simulation, and the variables h to a partial differential equations. The proposed approach may not be tractable in any situation, since it requires as an input in the modeling some prior biological functional knowledge and understanding of the elementary biological mechanisms. It rather arrives in a second step, when several pieces of understanding at different levels are gathered and need to be articulated in an integrated scenario.

• A first and already mentioned example is provided by the topological constraints experienced by a DNA molecule when its ends are anchored. Its linking number L_k is conserved and provides a global feature of the molecular assembly. The overall conformation of the DNA molecule is characterized by its writhe W_r, and $L_k - W_r$ corresponds to its total twist T_w. Any local modification within DNA molecule, e.g. a local structural change induced by some protein binding, should be compensated so as to ensure the conservation of L_k. Such coordination of local event by global topological constraints is essential in

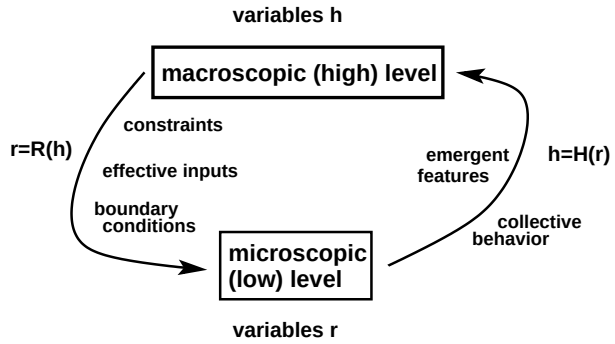

Fig. 1 Proposed self-consistent scheme for modeling biological systems. The expression $h = H(r)$ indicates in a compact way how the features h of emergent processes and superstructures depend on the effective parameters r describing the elements in a minimal way. Similarly $r = R(h)$ summarizes how the elements are affected by the higher-level entities. They are not the inverse one of the other. The terms "microscopic" and "macroscopic" denote two different levels of organization, e.g. individual and population level (e.g. bacteria and biolfilms), or molecular and organite or cell level (e.g. DNA and chromatin fiber).

procaryote transcriptional regulation (Travers and Muskhelishvili 2005). The same statement applies for the chromatin fiber in eukaryotes (Lesne and Victor 2006). The elastic coefficients of the chromatin fiber depend on the atomic details of its local architecture. Conversely, fiber supercoiling and balance between toroidal and plectonemic conformations induces mechanical constraints and strains down to the base-pair level.

- We have shown in (Bécavin et al. 2010) that RNA-polymerase activity within a condensed chromatin loop is possibly coordinated with a conformational change of downwards nucleosomes. RNA-polymerase activity generates supercoiling h in the chromatin fiber, described as a continuous elastic filament. In turn, supercoiling h induces a conformational change of some nucleosomes (individual state r), described as a transition between discrete states (nucleosome and reversome) and measured by the local density of reversomes. These transitions partly relax torsional constraints (i.e. induce a decrease of h) while allowing polymerase to process by providing a permissive substrate (polymerase can pass through a reversome), which in turn increases h again. Quantitatively, the self-consistent scheme bridges on the one hand the results of *in vitro* micro-manipulations (Bancaud et al. 2007) and modeling of the nucleosome structure (Zlatanova et al. 2009) with, on the other hand, the activity of RNA-polymerase, generating torsional constraints and requiring to encounter a permissive state of the nucleosome. The mediation is achieved by the coordinating influence of fiber supercoiling, and described using a partial differential equation for the propagation of torsional constraints in the chromatin fiber (Bécavin et al. 2010, Lesne et al. 2012).

- The proposed scheme has been implemented in (Malo et al. 2010, 2012) to construct an integrated scenario of metastatic escape based on partial in

vitro experiments. The principle is to bridge a system of ordinary differential equations describing in a minimal way the physiological switch of the cell between a proliferating and a migrating state, with a multi-agent simulation for the growing tumor, i.e. a population of cells. The coordinating intermediary is here the state-dependent secretion by the cells of proteins able to collectively modify the surroundings and induce a change of the cell physiological state.

• A last example, quite similar to the previous one, is provided by bacterial biofilms (Deygout et al. 2011, work in progress). Emergent properties of the bacterial population and, above all, the biofilm it secretes may exert feedbacks onto the very behavior of individuals, which conversely control the biofilm composition and geometry. The modeling challenge is to bridge a bottom-up approach, in which some emergent features are derived from a microscopic model, for instance an IBM simulation, with a top-down approach in which some global features control the IBM evolution. This scheme seems similar in spirit to the multiscale simulation proposed by (E and Engquist 2003; E et al. 2009) in hydrodynamics. However, the key difference is that top down constraints cannot be computed using some mean-field argument or more refined closure relations. They originate from a new entity, the biofilm, which does not reduce to the average or collective behavior of the bacterial population, but involves their secretion of a highly structured extracellular matrix. In principle, it could be possible to include the secretion of the bacteria and its regulation (and several other relevant physiological processes) in the IBM. In practice, a realistic simulation of a biofilm involving all the relevant physiological details for the bacterial cells is simply out of reach computationally, and would anyway raise huge difficulties regarding the robustness of the results. We need to recourse to a short-cut, namely devising a minimal model for a bacterial cell, involving only a few effective parameters that at the same time summarize the individual features controlling the biofilm properties and reflect the response of a bacterial cell to the constraints exerted by the biofilm. The difficulty is the identification and computation of these parameters, which essentially involves experimental knowledge and possibly an auxiliary microscopic simulation.The challenge is to quantitatively account for remarkable behaviors such as species coexistence (whereas the macroscopic mean-field equations predict the persistence of a single species and exclusion of the other ones), functional differentiation, spatial segregation, and other complex features of the bacterial population within a biofilm.

Self-consistency implicitly evoke self-consistent equations of physics involving continuous variables or fields. However, in principle, the proposed self-consistent scheme could fit in a discrete setting, as shown also by the above examples involving transitions between discrete states. Part of the difficulty in bridging discrete and continuous views might well originates in our descriptions (Lesne 2007). The Sorites (heap) paradox, like the paradox of Achilles and the tortoise, can be nowadays solved by the mathematical notion of limit, which was unknown to the ancient Greeks. Passing to the limit allows one to reach at infinity an entity of possibly different nature than the steps leading to it,

e.g. continuous whereas the steps are discrete (continuous limit, involved e.g. in describing the DNA molecule, made of discrete base pairs, as a continuous elastic filament, Sec. 4), or conversely discrete whereas the steps are continuous (step-wise limit of a sequence of increasingly steeper sigmoidal curves). The notion of emergence can be formulated is such terms: as summarized by Anderson (1972), "more is different". Another clue is to place the description at the unifying level of probability distributions, either for discrete-valued or continuous-valued variables (see (Lesne and Benecke 2008a, 2008b) in which this framework has been developed for the features involved in eukaryote transcriptional regulation). However, another facet of the difficulty is more intrinsic, and refers to the articulation between digital and analog *informations*. It relates to the question of biological codes, namely information storing, transfer and processing specific to living systems, much discussed since Schrödinger (1944). Inspiring insights about this delicate and still largely open issue as well as a way to solve it in the context of prokaryote transcriptional regulation can be found in (Marr et al. 2008, Muskhelichivili et al. 2010).

10 Conclusion

The first challenge of a multiscale analysis is to articulate experimental data, knowledge and models available at various levels. The aims are to bridge observations at different scales, to confront observations with mechanisms envisioned at another scale, and to integrate partial models into a consistent and explanatory account of the biological function.

The second and far more difficult challenge is to unravel the multiscale logic of living systems. Strikingly, collective effects may as a whole exert a feedback on the very properties of elementary ingredients and endow them with new functionalities. A specific multiscale approach is thus required to capture the architecture of biological systems and their regulation. A main guideline is their inter-level consistency, reflecting that evolution occurred *jointly* at all the levels of their organization. I propose an approach that makes use not only of effective parameters encapsulating at a given scale lower-level details, but also of effective inputs encapsulating the influences and constraints coming from superstructures and higher-levels processes. It is then completed and writing the self-consistency of these reciprocal couplings and influences. This amounts to considering elements and elementary mechanisms within their higher-level context, instead of in isolation. In this way, reductionnist and holist views of biological systems are reconciled, which paves the way for systems biology.

I finally underline that the very notion of what is a model prevents from devising an "all-purposes" model and replacing the study of the system by questions addressed on this model. To be fruitful, a model has to be specific to the investigated issue. It should ignore degrees of freedom irrelevant *to this issue* and its characteristic scales. Similarly, a multiscale model should not intend to keep track of all details at all scales but only of the relevant details, whatever their scales, essential to the biological function and its regulation.

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