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# Macromolecular networks and intelligence in microorganisms

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## Abstract

Living organisms persist by virtue of complex interactions among many components organized into dynamic, environment-responsive networks that span multiple scales and dimensions. Biological networks constitute a type of Information and Communication Technology (ICT): they receive information from the outside and inside of cells, integrate and interpret this information, and then activate a response. Biological networks enable molecules within cells, and even cells themselves, to communicate with each other and their environment. We have become accustomed to associating brain activity – particularly activity of the human brain – with a phenomenon we call "intelligence". Yet, four billion years of evolution could have selected networks with topologies and dynamics that confer traits analogous to this intelligence, even though they were outside the intercellular networks of the brain. Here, we explore how macromolecular networks in microbes confer intelligent characteristics, such as memory, anticipation, adaptation and reflection and we review current understanding of how network organization reflects the type of intelligence required for the environments in which they were selected. We propose that, if we were to leave terms such as "human" and "brain" out of the definition of "intelligence", all forms of life – from microbes to humans – exhibit some or all characteristics consistent with "intelligence". We then review advances in genome-wide data production and analysis, especially in microbes, that provide a lens into microbial intelligence and propose how the insights derived from quantitatively characterizing

biomolecular networks may enable synthetic biologists to create intelligent molecular networks for biotechnology, possibly generating new forms of intelligence, first *in silico* and then *in vivo*.

## 1. Introduction

For centuries, mankind has grappled with the precise nature and defining features of intelligence. Debates have erupted over how to define and measure the extent of intelligence in parts of the biological (and non-biological) world. Alan Turing, for example, famously proposed a test for evaluating the performance of "artificial intelligence": namely, can it be distinguished from the performance of human beings by another human (Turing, 1950)? A number of studies have explored whether there are differences in intelligence between human populations (Neisser et al., 1996), whether animals (Thorndike, 1998) and even plants (Trewavas, 2002) exhibit intelligent behaviors, whether non-human artificial systems are capable of intelligence (Brooks, 1991) and, more recently, whether intelligence spans biological domains including even the simplest of microbes (Hellingwerf et al., 1995;Bruggeman et al., 2000;Hoffer et al., 2001;Ben Jacob et al., 2004).

Here, we return to the issue of intelligence in microbes. However, rather than launch an ontological, epistemological, or semantic inquiry, we instead focus on the scientific utility of assigning intelligence to the microbial world. We review how the mathematical perspectives of complex adaptive systems and recent data-intensive developments in systems biology offer insight and help structure this problem. Finally, we consider whether viewing microbes through the lens of "intelligence" can help us better describe their behavior, harness their intelligence to perform valuable actions and, in the end, possibly extend our understanding of the systems biology underlying the functions of the human brain.

### 1.1. What is "intelligence"?

The modern biological perspective on "intelligence", even at its most fundamental level, tends to associate it with the human brain. In this context, "intelligence" is a property of the human brain, or a feature that somehow emerges from its activity. Accepting that intelligence may not be exclusively a feature of the human brain, but rather it may be present – at least to a degree – in all creatures possessing brains or nervous systems, already helps refine the general features of intelligence. However, intelligence may not have to be associated solely with a certain biological organ, such as a brain or a nervous system. Brains and nervous systems may be highly adapted conduits for expressing and integrating multiple intelligent behaviors. Some of these behaviors may be exhibited by other complex adaptive systems present in living organisms that do not have a brain or nervous system. Actually, already in 1995, Hellingwerf suggested that the properties of some two-component systems in bacteria complied with the requirements to be put upon the elements of a neural network (Hellingwerf et al., 1995). More recently, the so-called biogenic approach of cognition is gaining momentum in focusing on the biological origin of cognition and intelligence and by providing arguments to abandon a strict anthropocentric perspective (Lengeler, 2000;Lyon, 2006;van Duijn, 2012). This is the central paradigm around which we base our analysis.

## 1.2. How does intelligence emerge?

A small molecule at room temperature cannot be intelligent; it cannot store information about its past with implications for its behavior in some future. Large macromolecules, such as proteins – larger than RNases (Anfinsen, 1955) – and polynucleotides, may store information and Gibbs free energy in metastable states. The interactions between their structural components can differ depending on the way they have been folded some time ago. The background for this difference between small and large molecules is that small molecules have a sufficiently small number of structural microstates (conformations) so that all of these are visited by the molecule at any time scale relevant for biochemistry (10 milliseconds): they are "ergodic" (Westerhoff and van Dam, 1987). Large molecules may not travel all their microstates within seconds. In principle, when they would be phosphorylated and again dephosphorylated they may remain for a while in a different signaling state (Kamp and Westerhoff, 1986). This same feature may be important for high energy states of chromatin in epigenetics, where relaxation after refolding of nucleic acids and protein complexes may take hours, if not days.

The presence of information in an object requires that the object be away from its equilibrium state at least for some period of time. This may be achieved transiently by bringing the object into a high free energy state, with the relaxation back to the equilibrium state being slow. It may also be achieved permanently by making the former process permanent at the cost of Gibbs free energy, such as in the terminal phosphoryl bond in ATP. More generally, in open systems, Gibbs free energy harvested from the environment can be used to maintain the informative non-equilibrium state. Such free-energy transductions require the nonlinear interaction of multiple components: they require complexity (Westerhoff and van Dam, 1987); and so does intelligence.

Therefore, *vis-à-vis* memory, intelligence is already a property of a complex system as a whole; it is not reducible to the system's parts in isolation. Intelligence emerges when system components interact. For example, the intelligence (or intelligent-like behavior) we observe inside a single cell emerges from interactions among thousands of non-intelligent macromolecules. The intelligent behavior of a microbial society is not simply the sum of the behavior of intelligent cells, but the property which emerges from the interactions amongst many of them. And the intelligence of the human brain emerges from interactions of nearly 90 billion neurons.

In practice, it is not a trivial task to grasp interactions leading to intelligence. Perhaps the simplest way would be to construct a graph of the network of interacting components (molecules, microorganisms, neurons), thereby defining the topology of the interactions. Experimentally, this may correspond to performing yeast two-hybrid experiments or antibody pull down experiments. However, we will show that this does not suffice to establish the basis of intelligence. It is not only the existence of networks that create intelligent behavior: a rock can be full of networking structures and bonds among its component molecules and ions, but it is not intelligent. Therefore, the second step in understanding the interactions that lead to intelligence is the characterization of the dynamics of these interactions. The nonlinearities in the interactions and their indirect and incomplete, yet nonzero, reciprocities are important.

Although we may already know many, perhaps close to all of the components of a living system, e.g. the neurons and their connections in the brain (Alivisatos et al., 2012;Ahrens et al., 2013) and the

macromolecules and their interactions within the cell, we have no clear view on how they contribute to the proper and intelligent functioning of the whole system. One reason is that the complete picture is too complex to be perceived fully by our human brain. Using computer simulation, we should be able to reconstruct the emergence of these properties in a computational model. But even then, it is debatable whether our brain, biased by its very human nature, will be able to appreciate all forms of intelligence, especially forms that are not similar to our human intelligence. This is part of the transcendental challenge of this paper: the identification in microorganisms of forms of intelligence analogous to existing forms of human intelligence , which could have potential implementations in synthetic biology and perhaps even in engineering.

## 2. Systems biology of intelligence: reconstructing the emergence of intelligence from component properties of the system

Systems biology could be defined as a science that aims to understand how biological *function* that is absent from macromolecules in isolation, *emerges* when the same macromolecules exist as components in the *system* (Alberghina and Westerhoff, 2005;Westerhoff et al., 2009). The concepts of System, Function and Emergence are central in this context.

It goes without saying that the notion of function plays an important role in (systems) biology. Yet, the concept of function is, more often than not, rather ill-defined. Because the word "function" has strong teleological connotations, many biologists hasten to say that they invoke neither purpose nor intention when they are using the notion of function. On the other hand, the subtleties in reasoning that go with these notions are often overlooked (Wouters, 1999;Looijen, 2000), not in the least because the term function is used in various ways: "function" is a multifunctional term, as it were. Here, we will adopt the account of Wouters, who distinguished four principal kinds of biological function (Wouters, 1999). In short, he argues that the term "function" is used to refer to: (i) function as activity; (ii) function as role; (iii) function as advantage; and (iv) function as selected effect (Wouters, 2003; 2013). Mahner and Bunge also arrived independently to a similar set of functions (Mahner and Bunge, 2001). Considering the "cognitive" functions that are discussed in this study (decision making, robust adaptation, association, anticipation, self-awareness and problem solving), the first three functions are at stake, as this functional biology study is about cognitive capabilities of microbes that currently dwell on the Earth. The fourth kind is used in evolutionary biology and it features in historical evolutionary explanations.

The attribution of functions is important for giving functional explanations, which also come in different flavors, two of which are particularly relevant for this study: mechanistic explanations and design explanations. Mechanistic explanations analyze a system into a number of functional components, describe how these components are arranged and how their activities are organized in time, and relate how the features to be explained result from the properties of the interacting parts given their spatial and temporal organization (Boogerd et al., 2013). Mechanistic models are mathematical models of the action of networks of cellular reactions involving transport, metabolism, signal transduction or gene expression. However, mechanisms only explain how the features are brought about (how it works). To fully understand why the mechanism that produces the features to be explained is a good way to bring about those features, i.e. why alternative forms of organization would not work, or would work less efficiently, design explanations (why it works the way it works, and not differently) are required (Wouters, 1995;Wouters, 2007). They typically contrast the actual

form of organization (design) with a conceivable alternative, identify the features of the organism that depend on the form of organization being the way it is rather than the alternative, and account for these functional dependencies in terms of a certain kind of invariances ("laws").

A human brain comprised by neurons, a microbial community comprised by different species and individual organisms or an individual cell comprised by molecules are all semi-open systems. They all selectively interact with their environments by way of mass and energy exchange, where the decrease of free energy in the environment is coupled to the increase of the order of the biosystem itself (decreasing its own entropy), or with the maintenance of the biosystem against the activity of the many processes that tend to dissipate it (Westerhoff and van Dam, 1987). Systems of artificial intelligence are semi-open as well. They all need an external energy source to maintain their existence. In other words, there is always a flow of mass and energy through the system, and then a certain function emerges.

The Function we are interested in is Intelligence. Intelligence in fact consists of many functional features that allow the system to adapt to the environment and may even become the main system-forming factor. For example, the brain might evolve in the direction of being intelligent and artificial intelligent systems might be designed by human intelligence with the specific goal to be intelligent.

Together with other functions of the system, intelligence emerges from interactions of system components. As an emergent property, it satisfies three theses, as expounded by Stephan: (i) physical monism; (ii) synchronic determinism; and (iii) systemic (organizational) property (Stephan, 1999). The thesis of physical monism restricts the nature of the system's elements and states, so that the system consists of only physical entities and interactions, denying any supernatural influences – this is how we describe our system *ab initio*: we neglect all supernatural influences *de juro*. The thesis of synchronic determinism restricts the way systemic properties and the system's microstructure are related to each other and states that there can be no difference in systemic properties without changes in the structure of the system or in the properties of the components: features of intelligence are underlined exactly by the changes in the system (firing between neurons, chemical reactions between molecules, electrical current between components of a computer); in other words, differences in systemic properties should be measurable at least in principle and, with the advent of genomics and the other X-omics, also in practice. It is noteworthy that this thesis also implies that the reverse of this statement is not valid: a change in the microstructure or its properties does not necessarily yield a change in the system's behavior/properties. The thesis of being a systemic (organizational) property means that a property is not exhibited by elements in isolation; interactions must keep the elements out of their non-informative equilibrium state.

If emergence is weak, it simply satisfies just the three theses stated above. According to Stephan (Stephan, 1999; 2006), strong emergence would satisfy one additional criterion – the criterion of *irreducibility*. In general, there are three conditions for irreducibility, but it has been argued that for biochemical networks only one condition is relevant (Boogerd et al., 2005): if the properties of parts (say A, B, and C) in their relationship ($R_{ABC}$) *within* the system as a whole (together constituting an explanation of the systemic property at hand) do not follow from the properties of parts (A,B,C) or simpler subsystems (AB,BC,AC) in isolation, it is a strongly emergent property. It should be noted that in this definition of strong emergence, the prediction (deduction) base does not include systemic knowledge, such as the state of the state. Cognitive-like capabilities of a single microbial cell might then be irreducible in the sense that these properties cannot be deduced from the full knowledge of the behavior of the parts of the system in isolation or in configurations simpler than the one

prevailing within the whole system. In fact, all features of microbial intelligence described in this study are expected to be irreducible in this sense, and therefore strongly emergent.

It is worthwhile to compare our notion of strong emergence with the one featuring in the philosophy of mind. Here a mental property such as human intelligence is also considered as a case of strong emergence. However, the underlying rationale is often that it would be so because intelligence does not follow from full knowledge of the behavior of the parts and their interactions within the system. By implication, our point of view on microbial intelligence is basically different, because we assert that, in principle, any systemic property, including microbial cognitive properties, can be mechanistically explained, if the properties/behaviors of the parts and their relationship within the system are fully known, i.e. when full knowledge of the state of the system is available. For this reason, any microbial property can, in principle, be mechanistically explained and, thus, can also be reconstructed in mathematical models of the underlying mechanism provided that system knowledge is fully available. Properties that are declared strongly emergent – because of the limited prediction base – are still calculable, if the behavior of all relevant components and their mutual interactions within the system are available (Boogerd et al., 2005).

The limited prediction base of strong emergence provides the opportunity to rank emergent systemic properties according to the strength of emergence, which can be clarified as follows: in principle, every single component of the system, albeit indirectly, interacts with all other components. Let us consider an example of two abstract proteins A and B binding to each other inside the cell. The binding reaction between proteins A and B might depend on the presence of other proteins. For example, transporters and structural proteins forming intracellular compartmentalization keep proteins A and B together or separate them into different intracellular compartments. Other proteins (chaperones) might modulate the interaction directly by chemical modification of the interacting proteins. Binding between proteins A and B would also depend on intracellular pH. The latter is the result of the functioning of proteins regulating the uptake and pumping out of ions and different buffering molecules. In turn, many of those processes would be coupled with ATP hydrolysis and thus be dependent on the Gibbs free energy flux through the cell. Therefore, the way two components interact with each other depends to a variable extent on the state of the whole system. In other words, properties of components within the system are partially state dependent. The higher their state dependency, the higher is the degree of irreducibility (non-deducibility), and therefore the higher the strength of emergence (Kolodkin et al., 2012a;Kolodkin et al., 2012b).

The information of how components interact with and affect each other might be simplified and depicted as a pattern of major interactions and presented in the form of networks: metabolic networks, signal transduction networks, gene expression networks, anatomical networks, microbial ecological networks, etc. One can model these networks using various approaches. For example, one can determine the kinetic rules of how network components interact and express the rates of these interactions in terms of mathematical relationships, e.g. differential equations. Then, one can integrate all equations and solve them for the whole system. As a result, one may be able to simulate the dynamic behavior of the network and, thus, reconstruct its emergent properties *in silico*. For example, the response of the nuclear receptor network to the cortisol signal has been modelled in a kinetic ODE-based model (Kolodkin et al., 2013a). The essence of the network is rather simple: cortisol binds GR (glucocorticoid receptor) and activates it, activated GR inhibits (feedback loop) its own expression and activates (feedforward loop) the expression of another nuclear receptor, PXR (pregnane X receptor), which activates the expression of CYPA4 enzyme; the later degrades cortisol. Pulses of the cortisol imitating physiological stress were applied *in silico*. Unexpectedly, the

intelligent properties of the physiological network emerged in the computer model; for instance, the modelled system was able to learn from previous stress and anticipate the next cortisol pulse.

The example above shows how intelligent behavior can emerge from just one feedback and one feedforward loop. In reality, the network can be much more complicated and contain many such loops. Based on the architecture of neurons (including synapses and dendrites), feedback and feedforward loops are crucial for understanding the functional connectivity in the brain that is usually modelled by the artificial neural networks (Rolls and Treves, 1998). Neural networks are mainly classified into two groups, i.e. (i) the feedforward neural networks (FFNNs) where data is propagated from input to output under "combinatory machines", e.g. radial basis function (RBF), multilayer perceptron (MLP), self-organizing map (SOM); and (ii) the recurrent neural networks (RNNs). Several important feedforward loop motifs are identified in both neuronal connectivity networks and transcriptional gene regulation networks (Milo et al., 2002), despite these networks operating on different spatial and temporal scales. This similarity in motifs may reflect a fundamental similarity in the evolved designs of both types of networks: to reject transient input fluctuations/noises and activate output only if the input is persistent, a so-called persistence detector (Alon, 2007). In addition, a multi-input feedforward structure is identified in the neuronal network of the nematode *C. elegans*, which serves as a so-called coincidence detector: the output is activated only if stimuli from two or more different inputs occur within a certain period of time (Kashtan et al., 2004;Alon, 2007). Another biological example appears in the retina, where a hierarchical feedforward cortical architecture is used for the pre-processing of visual information (Sanger, 1989). A pure FFNN is rarely expected in the human brain neural system, but has produced successful practical applications. On the other hand, recurrent neural networks are more biologically meaningful (i.e. self-organizing dynamic systems) and can describe complex nonlinear dynamics, by including both feedforward and feedback structures. Nevertheless, very few real applications have been studied based on RNNs. Until recently, RNNs have been employed to study short-term memory and brain-like memory (Salihoglu, 2009). This is because RNNs allow the output of a neuron to influence its input, either directly or indirectly, via its effect on other neurons. This allows the network to reflect the input presented to it, but also its own internal activity at any given time.

In intracellular macromolecular network organization, a distinction has been made between dictatorial and democratic hierarchies, where only in the latter case the metabolite concentrations close to the systems output are able to influence gene expression (Westerhoff et al., 1990;Snoep et al., 2002). The two types of hierarchy may affect FFNNs and RNNs, respectively.

Learning and memory are two important features of "intelligence" and are identified as two competing processes. The former assimilates new information and requires flexibility in the network that can produce complex dynamics, while the latter retains old information and requires stability in the network with sufficient storing capacity. Tradeoffs between the two can be modelled and observed using neural networks. A recent study (Hermundstad et al., 2011) has investigated the links between the neural network architecture (e.g. parallel and layered networks) with the functional performance of the tradeoffs through feedforward neural networks. Another study (Salihoglu, 2009) indicated that classical feedforward networks with gradient descent learning algorithms would not be sufficient to describe the complex memory and learning dynamics, because real brain dynamics (e.g. in memory) are much more complex than fixed point attractors, but can be characterized by cyclic and chaotic regimes. Hence, the classical feedforward networks with gradient descent learning algorithms may not converge when complex nonlinear dynamics (e.g. bifurcation) exist and the RNNs would be a more appropriate choice for describing a memory-like structure. Also, feedback

structures can increase network stability and exhibit the paradoxical property of near-perfect adaptation, where many properties of the "system" can remain constant when the system is subject to an environmental challenge, through a strong change in (adaptation in) other properties of the same network (He et al., 2013).

These were some examples of how one can reconstruct and also understand the emergence of intelligence using information on component relationships, even when intelligence is a very strong form of emergence. In the next section, we will go into detail with examples of microbes exhibiting specific characteristics of intelligence (Figure 1).

## 3. Manifestations of intelligence in the microbial world

### 3.1. Decision making

Decision-making in humans is a vital process undertaken on a daily basis. It is a complex process that involves the coordinated activity of an extended neural network including several different areas of the brain. Making a decision requires the execution of several subtasks, such as outcome appraisal, cost-benefit analysis, and error perception, before finally selecting and implementing the optimal action. These processes can also be influenced by several factors such as personal preference, reward evaluation, reinforcement learning and social cooperation (Assadi et al., 2009;Gleichgerrcht et al., 2010). In the microbial world, decisions are made by monitoring the current state of the system, by processing this information and by taking action, with the ability to take into account several factors such as recent history, the likely future conditions and the cost and benefit of making a particular decision. At the population level, microbes are also capable of hedging their bets, by having individuals of an isogenic population in different states even when experiencing the same environmental conditions, and they are also able to make collective decisions that cause the entire population to respond in a particular way. Microbes are able to make decisions based on different criteria of information and also to perform the decision-making using different mechanisms, utilizing different types of molecular networks.

It can be argued that even simple biological systems like viruses are, under certain conditions, capable of decision-making when interacting with their host. A well-studied example is the bacteriophage lambda lysis/lysogeny decision upon infection of *E. coli*. The decision is regulated at the genetic level by a bistable switch, formed by mutual repression (Wegrzyn and Wegrzyn, 2005). The decision is made based on the conditions of the host cell and the number of phages present. However, stochastic effects are also thought to play a role, either through stochasticity in the expression and regulation of the lambda switch system (Arkin et al., 1998) or through differences between host cell environments prior to infection (St-Pierre and Endy, 2008). The fact that microbes experience stochasticity, due in part to low molecule numbers and the probabilistic nature of molecular interactions, adds layers of complexity to the decision-making process, for example the need to discriminate between signal and noise. With relatively-recent technological advances, experimental measurements of stochasticity are more readily obtainable and it has been found to affect some decision-making systems. This should be of no surprise, as stochasticity is at the basis of all time dependent processes; it is only the reproducibility that comes with high molecule numbers or linearity that removes stochasticity from observation (Westerhoff and van Dam, 1987).

One of the earliest known systems where a microbe makes decisions is that of ammonia transport and

assimilation in *E. coli* (van Heeswijk et al., 2013). The ammonium transporter (AmtB), the ammonium assimilating enzymes glutamate dehydrogenase (GDH) and glutamine synthetase (GS), and the helper enzyme glutamate synthase (GOGAT) are the main players in ammonium transport and assimilation at low environmental ammonium availability. A decision needs to be made between high-cost, high-accumulation transport by AmtB, low-cost, low-affinity assimilation by GDH, and high-cost, high-affinity assimilation by GS/GOGAT. In making this decision, *E. coli* has to deal with various costs and benefits at the same time: (i) maintaining intracellular ammonium at levels sufficient for growth; keeping in check energy costs (ii) of transport and (iii) of assimilation; (iv) minimizing a futile cycle generated by ammonium-ammonia movement across the membrane; and (v) preventing or minimizing the wastage of ATP by the simultaneous action of biosynthetic GS and degradative GDH. *E. coli* avails of a complex hierarchical regulatory network to make this delicate decision, involving gene expression, signal transduction, metabolic regulation and transport at the same time (Kahn and Westerhoff, 1991;Bruggeman et al., 2005;Boogerd et al., 2011;van Heeswijk et al., 2013).

Many prokaryotic cells are able to move through liquids or over moist surfaces by using a variety of motility mechanisms (swimming, swarming, gliding, twitching, floating) and mostly use complex sensory devices to control their movements (Jarrell and McBride, 2008). The decision of microbes to move towards nutrient sources or away from toxic compounds is another one that seems intuitively "intelligent". The most studied system is that of chemotaxis in *E. coli*, with common features in other prokaryotes and eukaryotes. In order to make this decision, the cell monitors the environment by means of multiple receptors in the cell membrane. The information of the ligand binding to the receptor, and the processing of this information inside the cell, is achieved by means of a signaling pathway involving methylation and phosphorylation, as opposed to the genetic switch seen in the lysis/lysogeny decision (Bourret and Stock, 2002). The level of phosphorylated CheY, the downstream protein of the signaling pathway, determines which movements the cell undertakes: when phosphorylated CheY is bound to the flagellar motor (i.e. when an attractor ligand is present) it rotates counter-clockwise, resulting in a straight swimming movement; in the absence of phosphorylated CheY the unbound flagellar motor rotates clockwise, resulting in a tumbling motion. Using this mechanism, organisms make a biased-random walk, with the length of the periods of straight swimming dependent on the signal, resulting in movement towards or away from different stimuli.

*Pseudomonas aeruginosa* has been shown to make its decisions about which of its two siderophore-dependent iron acquisition systems to use when faced with iron limitation based on the cost-to-benefit ratios of the two options (Dumas et al., 2013). The two mechanisms have different costs and benefits to the cell: one mechanism, using the pyoverdine siderophore, has a high iron scavenging efficiency (since pyoverdine has a high iron affinity $Ka$ $10^{32}$ $M^{-1}$), but comes at a high cost, requiring the expression of at least 14 genes, hence utilizing high amounts of nucleotides, amino acids, ATP, and other cellular resources. The other mechanism, using the siderophore pyochelin, has a lower cost to the cell, because of a simpler biosynthetic pathway consisting only of 7 genes, hence requiring the utilization of few cellular resources, but has a much reduced efficiency of iron-acquisition (since its affinity to iron is relatively low, $Ka$ $10^{6}$ $M^{-1}$). The processing of the information required to make a decision is achieved by the finely-tuned parameters of the two systems' feedback loops that enable them to exhibit different sensitivities. The parameters of the feedback loop for the high-cost, high-efficiency system limit its use to extreme iron limitation conditions and the parameters of the feedback loop for the low-cost, low-efficiency system enable it to be utilized in more moderate iron limitation, thereby optimizing the cost-benefit ratio.

The decision of *Bacillus subtilis* to become transformation-competent (i.e. able to take up DNA) is taken at an individual level, but the mechanism by which it occurs results in a portion of the population being in a state of competence. The decision-making regulatory system is a bistable switch that functions in a finely-balanced manner around the critical threshold, which, once passed, leads to the decision to become competent (Maamar et al., 2007;Leisner et al., 2008). Due to the system operating close to the threshold, stochastic fluctuations in the levels of one protein, ComK, are able to push the cell over the threshold to begin the transition to competence (Maamar et al., 2007). As this is based on stochasticity, it will only occur in a portion of the cells in a population. This expression of different phenotypes by an isogenic population of cells in the same environment is considered to be a bet-hedging strategy (Veening et al., 2008). Although each individual may not be in the optimal state for the given conditions, the population as a whole gains an advantage by being more adaptable to changes and having increased chances of survival of the population.

Through the above examples of decision-making in microbes, it can be seen that there are several common features that are analogous to processes involved in human decision-making. Although the network components may vary (gene-expression regulation, signaling pathways, metabolism, transport), the networks involved and the parameters controlling their interactions allow the microbes to monitor their environment, process the information and react, effectively making a decision in an "intelligent" manner by taking into account such factors as the cost-benefit ratio and population survival strategies.

### 3.2. Robust adaptation

An important feature of "intelligence" in microbes is the robust adaptation to changes in environments, for example homeostasis, as well as adaptive tracking or following of the sources of nutrients and escaping from harmful compounds, for example through bacterial chemotaxis. Almost all these adaptation mechanisms involve feedback or feedforward regulation structures (or motifs). This can be relevant for signaling, gene regulatory and metabolic networks, where homeostasis can be introduced via fine-tuning between rate constants in feedback and feedforward motifs. Relatively long term adaptations, which sometimes happen throughout the microbe's lifetime, often involve changes in genetic expression, such as gene mutations, transcription/translation activities or rewiring of gene regulatory networks – for a review see (Brooks et al., 2011). Examples are adaptation to salt conditions, temperature or asymmetric cell division. Short term adaptation requires regulations mediated by (i) protein-protein interactions and covalent modifications (e.g. phosphorylations) in signal transduction pathways; or (ii) allosteric or more direct substrate-product effects in metabolic networks. Of all the adaptive regulations, robust perfect adaptation is of particular interest. It describes an organism's response to an external perturbation by regulating some of its state variables precisely to their original values before perturbation. For example, perfect adaptation has been reported in bacterial (e.g. *E. coli*) chemotaxis (Berg and Tedesco, 1975;Alon et al., 1999;Yi et al., 2000;Hansen et al., 2008), osmotic-stress adaptations (Muzzey et al., 2009) and MAP-kinase regulation (Hao et al., 2007;Mettetal et al., 2008). Such perfect adaption behaviors are thought to be introduced through a time integral on the "controlled variable" in the network, which corresponds to a specific control system structure, i.e. an integral feedback control (Csete and Doyle, 2002). A recent *in silico* study (Ma et al., 2009) identified an alternative topology that can also ensure perfect adaptation through an incoherent feedforward structure, where a positive regulation cancels out the effect of a simultaneous negative regulation, hence the overall output is insensitive to the input signal. Because it should be difficult experimentally to discriminate between perfect and strong

adaptation and because at least some of the proposed mechanisms for perfect adaptation require biochemically unrealistic features, such as zero order degradation of proteins (He et al., 2013), the evidence for truly perfect adaptation should perhaps be revisited; the adaptation may be less perfect than stipulated, with robustness being strong, but limited. Here, it would help if this robustness were quantified (Quinton-Tulloch et al., 2013). In non-robust "proportional" (He et al., 2013) regulations, the appearance of a specific signal or environmental condition can be a direct indicator/predictor of a particular response. The feedforward regulatory mechanism is then introduced to respond directly to the signal rather than to the disturbance. Feedforward regulatory structures were observed in gene regulatory networks in the regulation of membrane lipid homeostasis (Mangan and Alon, 2003;Albanesi et al., 2013), in bacterial photosynthesis genes for optimal free-energy supply (Mank et al., 2013), and in the heat shock response in *E. coli* (Shudo et al., 2003).

Different regulation mechanisms in living cells often occur at multiple levels simultaneously with a hierarchical structure (Westerhoff, 2008). For example, in a microbial metabolic network, the regulation of a reaction rate can be achieved by the modulation of (i) enzyme activity through a substrate or product effect, or through an allosteric effect, i.e. metabolic regulation; (ii) enzyme covalent modification via signal transduction pathway; or (iii) enzyme concentration via gene expression, gene-expression regulation. Such multiple-level regulations correspond to different control loops in a control system, which may ensure the robustness versus perturbations at various frequencies as employed in engineering system design. Let us consider an unbranched metabolic pathway, with the first enzyme inhibited by the end-product via both allosteric/metabolic and gene-expression regulation. If the flux demand on the end-product module increases rapidly, the concentration of the end-product decreases rapidly. Often, as a result of the allosteric effect of the end-product directly on the first enzyme, the activity of that first enzyme increases quickly too. This metabolic control of enzyme activity is a fast "actuator" of the system. However, if there is a further increase in the flux demand, the first enzyme may lose its regulatory capacity since its activity may be approaching its maximum capacity ($k_{cat}$). At this stage, the system has a second "adaptation" through gene expression that is slow but leads to an increase in the concentration of the first enzyme, which then decreases the direct stimulation of the catalytic activity of the first enzyme. The regulation of the first enzyme is then bi-functional in dynamic terms (Csete and Doyle, 2002): the metabolic regulation rapidly buffers against high frequency perturbations, but possibly with small amplitude or capability, while the gene-expression regulation is slow to adapt, but may be able to accommodate very large constant perturbations (ter Kuile and Westerhoff, 2001).

When interpreting metabolic and gene-expression regulation separately as specific "control system structures", the former was recently identified as more of a "proportional control" action (Yi et al., 2000;El-Samad et al., 2002) with limited range and the latter as more of an "integral control" action with potentially a wider range, but acting more slowly (He et al., 2013). Such control engineering interpretations can also be linked with classical Metabolic Control Analysis (MCA) (Fell, 1997) and Hierarchical Control Analysis (HCA) (Kahn and Westerhoff, 1991). The relatively fast metabolic regulation is related to the direct "elasticities" of MCA, while the slow gene-expression regulation corresponds to the indirect "elasticities" of HCA (He et al., 2013).

### 3.3. Association and anticipation

Associative learning allows one to model how two or more features in the world co-vary and respond accordingly. This type of learning provides context, in the sense that it specifies how several features

in the environment, or within cells, change together. It implies that the learner has a mechanism to encode mutual information. In humans and animals, this type of learning has been associated with experimental settings where, for example, a subject is conditioned (often through an auditory or visual cue) to activate unconditioned responses (like salivation) after presenting the subject with a conditioned stimulus (e.g. a bell) simultaneous to the unconditioned stimulus (e.g. dinner) that usually elicits the unconditioned response. After a period of learning the association, the unconditioned response (salivation) can be achieved in the absence of the unconditioned stimulus (simply ringing the bell). Conditioned behaviors like this have been well studied in humans and other animals since the pioneering work of Ivan Pavlov (Pavlov and Anrep, 1927). Recently, the molecular mechanisms responsible for encoding these behaviors in neurons have been defined (Maren et al., 2013). In general, these mechanisms rely on the plasticity of neurons to reinforce electrochemical couplings, such as changing the localization and abundance of glutamate and NMDA receptors at synapses (Nakazawa et al., 2002;Rumpel et al., 2005).

Associative learning allows learners to structure dependencies that exist in the world. Pavlov's dog, for example, salivates because of the linkage the dog has learned between bell and dinner; even though the association is entirely manufactured in this case. Outside of contrived laboratory conditioning, associative learning occurs when environmental variables are physically coupled, or somehow co-vary non-randomly. For example, the increase in the level of light (photons) at sunrise, signals associated changes in the environment, such as increase in temperature, change in $O_2$ availability, etc. Organisms leverage these physical associations to better adjust their physiology in specific environments (Bonneau et al., 2007), to employ easily-measured proxies as indications for other phenomena (like the bell for Pavlov's dog) and, in some cases, even use the cues themselves to prepare or "anticipate" subsequent alterations to the environment. Investigators have asked recently whether organisms like microbes, which do not have nervous systems, can also exhibit associative learning and anticipation.

Several experimental studies and modeling efforts have suggested that, indeed, microbes can learn associations, both as communities and individually. Studies, furthermore, suggest that gene regulatory networks can encode associative learning. One of the most comprehensive examples of this phenomenon comes from a study of the bacterium *E. coli* (Tagkopoulos et al., 2008). As a microbe that lives both in the soil and the guts of mammals, *E. coli* has to adjust its physiology to environments that vary with respect to important biological parameters, such as temperature and oxygen availability. Since many of these environmental parameters do not change randomly, but rather in coupled ways (e.g., increase of temperature in the oral cavity and corresponding decrease in oxygen availability in the gut), *E. coli* is able to take advantage of this predictable physical association to direct its physiology accordingly. In this study, the authors demonstrated that transcriptional responses in elevated temperatures are highly similar to those observed in oxygen perturbation experiments, even though the second stimulus is absent (much in the same way that Pavlov's dog can be stimulated to salivate simply by ringing a bell). More impressively, they showed that *E. coli* can "re-learn" these associations. Relative to ancestral *E. coli*, evolved strains grow better in environments where temperature and oxygen are decoupled (in this case inverted). This study demonstrates (1) that microbes have both the capacity for associative learning, and (2) that the learned associations are plastic. A similar study in yeast suggested that previous lifestyle plays an important role in adaptation to severe stress, re-emphasizing the existence of associative learning in microbes (Berry et al., 2011).

It is important to note, however, that time-scale for this "learning" is on the order of evolution processes and most likely involves genetic changes. This has an analogy of the development of

"fixed" hard-wired neuronal connections in a brain or cultural learning in human society. In the example, it took many generations for *E. coli* to learn about the altered association between oxygen and temperature and, presumably, much longer for the natural situation to be canalized. Critical questions for future studies will include whether gene regulatory networks encode associations that are capable of being learned within the lifetime of an individual bacterium; a case in point was made for ammonia assimilation in *E. coli* (Hellingwerf et al., 1995;Bruggeman et al., 2000). A recent modeling study suggested that gene regulatory networks composed of bistable elements with stochastic dynamics can exhibit associative learning, although the number of learnable associations may scale as the square root of the number of bistable elements (Sorek et al., 2013). Similar results have been obtained in the context of chemical networks (McGregor et al., 2012) and other transcriptional networks (Carrera et al., 2012). Additional experiments, however, are required to evaluate whether the dynamics of cellular networks with multiple stable states are sufficient to encode and retrieve contextual associations. Hellingwerf showed learning behavior should be possible in realistic mono-stable *E. coli* networks (Hellingwerf et al., 1995).

Among microbial populations, associative learning seems to be commonplace. Mechanisms and examples of associative learning in microbial communities have been discussed extensively elsewhere (Ben Jacob et al., 2004;Xavier, 2011). Typically, associative learning in microbial populations involves some sort of social communication (such as quorum sensing, discussed in section 3.4). This type of networked communication is highly plastic and eminently reminiscent of neuronal activities. Other examples of association and anticipation in the microbial world are exhibited by pathogenic bacteria such as *Pseudomonas aeruginosa*, which is an important human, animal, and plant opportunistic pathogen and, perhaps, the bacterial species that has most genes devoted to regulatory purposes (Stover et al., 2000). In the context of human digestive tract infections, this bacterium senses several compounds released by the host tissues, such as interferon, opioids, and metabolites like adenosine, which are all released into the intestinal tissues and lumen during surgical injury, ischemia and inflammation. In addition, it senses the extracellular levels of phosphorus, which decrease severely when the patient condition is deteriorates. Hence, when the bacteria senses high concentrations of host-released compounds together with a decrease in phosphate levels, it anticipates the vulnerability of the patient and turns on several virulence determinants that frequently lead to lethal sepsis (Zaborin et al., 2009). Microbial cell-cell communication allows cells to anticipate a lack of nutrients and to stay at the stationary phase, preventing the complete depletion of nutrients. Recently, it was demonstrated that in the genus *Burkholderia,* quorum sensing allows the activation of cellular enzymes required for production and secretion of oxalic acid, which serves to counteract ammonia-mediated alkaline toxicity during the stationary phase, hence anticipating a stress situation and triggering a preventive strategy that helps cells better adapt to the oncoming harsh environmental conditions (Goo et al., 2012).

The capacity for associative learning among microbes may be one of the reasons why we are able to reverse engineer them. Since microbes do not respond to stimuli independently, but rather their internal networks direct common responses to diverse but related environmental signals, regulatory networks in microbes can be reconstructed by simply measuring their response across a broad range of conditions. Gene regulatory networks, for example, can be inferred in three simple steps: (i) perturb cells across a broad range of relevant conditions; (ii) measure their transcriptional response in each environment; and (iii) cluster similar gene expression patterns observed reproducibly across environments. Mining for genetic similarities among genes sharing a particular expression pattern, such as common *cis*-regulatory elements in their promoter regions, in turn helps link these transcriptional modules to some of the molecular mechanisms responsible for regulating them. In practice, such approaches allow the construction of gene regulatory networks directly from

transcriptome measurements (Reiss et al., 2006) [Brooks, Reiss *et. al*, Submitted]. It should be recognized, however, that the networks thus reconstructed are incomplete, as they forego the signal transduction and metabolic networks that are part of the actual regulation (ter Kuile and Westerhoff, 2001).

### 3.4. Quorum sensing and self-awareness in microbial populations and communities

Quorum sensing is a widespread type of bacterial cell-cell communication between individuals of the same or different species (Waters and Bassler, 2005;Lee et al., 2007;Hosni et al., 2011), aiming at the estimation of population size. The accepted paradigm for this kind of communication is that individual cells are steadily producing and releasing several kinds of small diffusible molecules (signals), called auto-inducers, and that in parallel each cell has the ability to sense the presence of those molecules, by means of receptors/transcriptional modulator proteins that bind the auto-inducers and, once complexed with them, trigger a global transcriptional response that leads to crucial changes in the expression of several phenotypes and behaviors. An important property of quorum sensing communication is that the response is only achieved after one specific signal (cell number) threshold is exceeded, by means of a positive feedback loop mediated by the auto-inducer production, since genes for the enzymes that biosynthesize the signals are under their own control. There is a plethora of behaviors and phenotypes controlled by quorum sensing systems; among them: light production by several species of the *Vibrio* genus, competence (i.e. the ability to uptake and incorporate foreign DNA), biofilm formation, synthesis of secondary metabolites and the production of virulence factors.

Self-awareness can be described as the ability to recognize oneself as an individual separate from the environment and other individuals. Quorum sensing provides the entire bacterial network with the ability to recognize and adjust itself in a collective level, once a specific population threshold is exceeded. This is specific for all individuals of a certain organism and even strain. Equating individual with clonal population, quorum sensing appears to generate self-awareness.

Signaling specific to some environmental cues is interwoven with quorum sensing signaling; for example in *Pseudomonas aeruginosa*, the iron availability signal network and the quorum sensing system communicate and influence each other (Juhas et al., 2004). In addition, bacteria can sense quorum sensing signals of other species (Federle, 2009) and act in accordance with the population sizes of competing or mutualistic species and even of eukaryotic or pluricellular organisms, such as their hosts (Bansal et al., 2010;Hosni et al., 2011;Ma et al., 2012). Hence, they have the ability to distinguish themselves from other similar networks in other species. Most of the bacterial cell-cell communication described to date exclusively involves the release of autoinducers to the extracellular medium and the sensing of those molecules by other cells; phenomena that depend on the diffusion of the signals and therefore lack directionality. Since, in a well-mixed environment such as a stirred liquid culture of planktonic cells, one cell can sense the auto-inducer produced by any other cell, communication among network components should be uniform. This is in contrast to communication among molecule types in signal transduction networks and among cells in neuronal networks. In the latter cases, each member interacts directly only with a limited set of other network components, creating clusters and functional domains that, together, form a structured network with non-trivial topological features and a higher-than-random complexity. The situation changes in more realistic environments, such as in bacterial biofilms, which are known to be the preferred lifestyle of several bacterial species (Costerton et al., 1995). Those biofilms can be composed of a single bacterium species, but more often are complex ecologies of single-cell organisms that may include hundreds of

different species of algae, bacteria, protozoa, fungi and viruses. They collectively generate and embed themselves in an extracellular polymeric matrix that provides structure and protection. In such environments, cell-cell communication could be more specifically performed among clusters of cells organized in different spatial and functional biofilm domains. Recently, the discovery of bacterial communication networks of multiple cells of *Bacillus subtilis* that are directly connected to others by bacterial nanotubes was reported (Dubey and Ben-Yehuda, 2011). These structures are able to mediate the exchange of non-conjugative plasmids, metabolites and even enzymes, and can be formed in an interspecies manner between *B. subtilis* and *Staphylococcus aureus* or even the phylogenetically more distant *E. coli*. The authors speculated that these kinds of networks may represent a major form of bacterial communication in nature. If so, they may constitute complex and intricate structured bacterial communication networks with high potential to exhibit intelligent behavior.

Some features of self-awareness can be manifested already at a lower level of social organization of microorganisms. Thus, bacteria of the same species are capable of assembling together and isolating themselves from other species. This advanced social organization would be reflected in cooperation; for example in swarming motility (coordinated translocation of many bacterial cells), in collective repairing of holes in biofilm, in collective capture and digestion of food, etc. Microorganisms can cooperate for collective aggression through the coordinated production of antibiotics. There are even "bacteria-altruists", who sacrifice themselves to become food for their brethren (Oleskin, 2009). However, at the opposite extreme, there also exist "microbe-cheaters", which can disrupt cooperative systems by acquiring a disproportionate share of group-generated resources while making relatively small contributions (Velicer, 2003).

Finally, it is worthwhile to note that philosophers of biology are also beginning to appreciate the remarkable microbial capacities for cooperation and communication and to shift attention from the macrobes to the microbes (O'Malley and Dupré, 2007a;O'Malley and Dupré, 2007b;O'Malley, 2013).

### 3.5. Problem solving

An essential feature of any intelligent system is that, in addition to storing information and to having the ability to incorporate new knowledge from its experiences, it must have the ability to use that knowledge to solve new problems. Generally, the more complex a problem a system can solve, the more intelligent it is considered. In this regard, some microorganism networks show problem solving abilities that can even match or surpass those shown by human beings: the slime mold *Physarum polycephalum* in its plasmodium configuration, which is a large multi-nuclear amoeba-like cell consisting of a dendritic network of pseudopodia, has the ability to connect two different food sources located at different points using the minimum-length pathway in a labyrinth, which optimizes its foraging efficiency (Nakagaki et al., 2000). Moreover, this organism is able to develop much more complex networks efficiently, and even to solve combinatorial optimization problems optimally, such as connecting several food sources simultaneously. The mold is able to create solutions with comparable efficiency, fault tolerance and cost to those of human infrastructure networks, such as the Tokyo rail system, but, unlike humans, the mold achieves optimal solutions solely by a process of selective reinforcement of the preferred routes and the simultaneous removal of redundant connections, without any centralized control or explicit global information. This striking mold ability was captured in a mathematical model, which the authors claim can provide a starting point to improve routing protocols and topology control for self-organized networks used for human transport

and communication systems (Tero et al., 2010). This is a perfect example of applied microbial intelligence with the potential to improve everyday human life conditions.

## 4. Learning from intelligence in the microbial world

Given the examples of the previous section, it is likely that, at least for some specific tasks, microbial "intelligence" can compare to human intelligence, and therefore microbial networks could be considered formally as "intelligent". Recognizing microbial intelligence can allow us to potentially modify microbial networks or to *de novo* develop new ones that are able to provide intelligent solutions to specific human problems. If intelligence (or components thereof) emerges from the dynamics of complex adaptive systems and the human brain is an evolved organ for the encapsulation of intelligent characteristics, it is possible that there are features of intelligence that remain undiscovered.

### 4.1. A deeper understanding of the microbial world

One important and exciting domain of synthetic biology is the manipulation and design of microbial metabolism for chemical production in the energy, biomedicine and food industry (Purnick and Weiss, 2009). Such design relies on effective control and adaptation of metabolism (e.g. pathway flux) in response to intracellular or environmental perturbations. In an engineered genetic-metabolic circuit, there are many parameters that can be used for design purposes. Promoter characteristics, such as tightness, strength or regulatory sites, can be engineered in the transcriptional control, and the engineering of ribosome binding sites or RNA degradation can be used to control the expression levels of proteins. Well-known examples are the genetic control of lycopene production in *E. coli* (Farmer and Liao, 2000) and the design of gene-metabolic oscillators (Fung et al., 2005;Stricker et al., 2008). Designing scaffold proteins in the protein-protein interaction domain has been studied for the control of metabolic flux (Dueber et al., 2009). Recent studies (He et al., 2013;Westerhoff et al., 2014) showed that although gene-expression regulation can increase the robustness of an intermediate metabolite concentration, it rarely makes the metabolic pathway infinitely robust. For perfect adaptation to occur, the protein degradation reactions should be zero-order in the concentration of the protein or the living cell should enter stationary phase after a period of growth. However, the former scenario is rarely observed biologically. Nevertheless, in some situations, protein degradation rates can be controlled by adding or removing a degradation tag to the gene sequence (McGinness et al., 2006). In this way, a relatively small degradation rate may be obtained in an engineered gene-metabolic network, and near-perfect adaptation behavior can be achieved with a quasi-integral control structure.

### 4.2. Microbial vs. human intelligence

Our paper collects various examples of the intelligent features discovered in the microbial world (Figure 1). Microbial intelligence emerges from the dynamic interactions among macromolecules. Intelligence is a strong form of emergence; its reconstruction requires information of state-dependent component properties. The more state-depend information we need, the stronger the emergence is.

The degree of state-dependency of the component property is determined by the presence of other components in the system affecting this property, on the flux of matter through the system and on the history of the system (Kolodkin et al., 2012a;Kolodkin et al., 2012b;Kolodkin et al., 2013b). In this context, we can scale and compare the strength of emergence of intelligence for different complex adaptive systems, e.g. for microorganisms, animals or humans.

Rough, back-of-the-envelope estimations suggest that the number of intracellular interactions affecting the state-dependent property of a certain molecule in bacteria could be tremendously high. For example, the ability of a single transcription factor to bind a response element might depend on the presence of tens of other transcription factors and their ligands, on hundreds of components involved in intracellular trafficking of all these ligands, on thousands of molecules providing ATP-convertible free energy for this trafficking and for receptor synthesis, and on plenty of other molecules that maintain a certain pH, viscosity and macromolecular crowding. By taking all of these into consideration, the number of intracellular interactions might become comparable with the number of neurons which affect the firing of another neuron. Thus, the strength of the emergence of intelligence that is raised due to interactions in an intracellular (e.g. microbial) network might be also comparable with the strength of the emergence of intelligence in the human brain. Does this mean that bacteria are as intelligent as humans? Still, we feel intuitively that the intelligence in microorganisms and in humans is different.

For instance, the physiological adaptive behavior of microorganisms is not stable and disappears when the environment does not support this behavior. Programs of adaptive behavior are imprinted on the population genome. When adaptation is lost, new training is required to regain this adaptation. Microorganisms exhibit some features of elastic behavior, but they do not have the conditional reflexes of higher animals. In an evolutionary context, in animals the elementary reflection of the environment is replaced by perceptive reflection and animals gain different forms of individually-adapted behavioral changes co-tuned to the changes in the environment. Animal activity toward objects develops depending on the objects animals have already dealt with. This correlates with anatomical changes; the cerebral cortex emerges in addition to basal ganglia that cause a crucial shift in animal behavior. Basal ganglia enable signal reception and turn on inherited behavioral programs. The cerebral cortex, in its turn, enables analysis and integration of external signals, reflection on external objects and situations, building up of new connections and, ultimately, development of the behavior that is based, not on the inherited programs, but rather on the animal's perception of external reality. With the development of the cerebral cortex, new forms of individual behavior based on objective reflection of the environment are formed.

Further development of the cerebral cortex takes place in humans. Aside from both inherited programs and individually gained experience, humans develop a third form of behavior: the ability to transfer collective experience from one human being to another. The transfer of collective experience includes the knowledge gained at school, at work, in life, etc. Animals are born with the inherited programs and enrich these programs through individual experience. Humans might be born with the poorest instinctive inborn programs, but can develop their mental processes, not only through personal experience, but also through learning from collective experience. Human individuals are able to communicate with each other and even, through literature, with their predecessors.

The development of human psychic activity is related to the further development of the cerebral cortex. Nevertheless, in the context of scaling the degree of the strength of emergence, the complexity of the human brain does not change immensely. Rather, the new behavior emerges from the changes in the design, and not from a tremendous increase of interacting components.

The degree of the strength of emergence of intelligence is higher in microorganisms' intracellular networks than in animal brains; the strength of the emergence in animal brains is comparable with the one in humans. Still, the intelligence is different. Thus, humans model microorganisms and debate about microbial intelligence, while bacteria are probably not even aware of us.

### 4.3. The way forward

Most aspects of human intelligence are also exhibited by microorganisms at least to some degree, except those that depend on reading and writing. The examples we presented regarding quorum sensing and problem solving were from multicellular networks. The question remains whether networks at any single, more molecular level, such as intracellular signaling, also exhibit most aspects of intelligence. It has been proposed that intracellular quorum sensing occurs during mitochondrial apoptosis (Brady et al., 2006). The hierarchy of regulatory networks involved in ammonia assimilation is a candidate for rich intelligent behavior. The molecular information is now so complete (van Heeswijk et al., 2013) that it may well be possible to develop the existing replica models (Bruggeman et al., 2005) into a full representation. These may then be used to determine the extent to which our present molecular network understanding suffices to demonstrate that these networks should be expected to exhibit almost all types of intelligent behavior (Hellingwerf et al., 1995;Bruggeman et al., 2000). This could then also help with experimental design driving subsequent experimental testing. Similarly, such mathematical representations may also be used to search for new aspects of intelligence that we, as humans, do not recognize as such, for example adjustable robustness, random creativity facilitated by deterministic chaos in the networks, productive noise thereby, and read-only memory. Many of these aspects may be useful for synthetic biology; a synthetic biology that will give rise to much more sustainable, productive systems.

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## 7. Figure legends

**Figure 1 Microbial intelligence vs. human intelligence.** Microbes exhibit similar characteristics of intelligent as higher organisms and humans, like decision making, association and anticipation, self-awareness and problem solving capabilities.