Molecules and Meaning: How Do Molecules Become Biochemical Signals?

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ABSTRACT

The objective of this paper is to reflect on how molecules can acquire macroscopic meaning (i.e., carry a message to macroscopic levels) in a context of biological evolution. First, the structure of molecules is explained in terms of form (molecular geometry), function (measurable or computable molecular properties), and fluctuation. Fluctuations in form and function create distinct molecular states, and the ensemble of all molecular states defines a molecular space (also known as a property space).

The second part examines molecules in a chemical context. The interplay between a chemical compound and its environment creates a complex system in its own right, as exemplified by solutions. A solute influences the solvent by affecting its organization and some colligative properties, while the solvent often has a marked influence on the solute by constraining its property space and so selecting some of its molecular states. Solutions may display emergent properties not existing in the separate components, e.g. chemical reactivity, implying that information has been created upon formation of the complex system.

The third part of the paper discusses the interaction of chemical compounds with biological media. In contrast to abiotic environments such as solvents whose degree of organization is comparatively low, biological media are characterized by a high degree of organization. Examples at the macromolecular level include functional proteins (receptors, enzymes, transporters, ...) or nucleic acids. When a molecule is recognized by such a macromolecule and interacts (binds) productively with it, a complex system is produced whose emergent property is the functional response, and which strongly constrains both of its components. The chemical is frozen into a single or a very limited

number of molecular states (induced fit), whereas the macromolecule is activated by a conformational change (e.g. an allosteric effect). Here again, emergent information appears in the complex. However, there is an essential difference with abiotic systems since the emergent information can now be translated into a functional biochemical reaction that in turn will be amplified into a macroscopic biological response. In other words, information emerging in the molecule-macromolecule complex is a signal that becomes meaning as it is recognized in the higher hierarchy of nested biological contexts.

1 PREAMBLE: SIGNAL MOLECULES

Chemical signals were the first means of long-distance communication evolved by living organisms, and they remain among the most effective and specific ones. Thus, pheromones are a major means of communication between conspecific organisms, whereas different species can interact via chemical signals that elicit attraction, repulsion, cooperation, etc. Chemical regulation within multicellular organisms is particularly well documented and involves hormones, neurotransmitters and other regulators. Drugs are another case in point, being messages sent to ailing cells and organs and aimed at correcting pathological states (Testa 1996, 1997). A list of signal molecules therefore includes:

- poisons and repellents (inter-species)
- attractors (inter- and intra-species)
- pheromones (intra-species)
- neurotransmitters, hormones, growth factors (intra-organisms)
- drugs

The overall molecular mechanism by which information is transmitted from organism to organism or from cell to cell is always the same, namely:

- Emitted signal molecules are recognized by and fit into specific receptors (The key enters the lock).
- The formation of the receptor-ligand complex alters the state of the receptor (The lock is turned).
- This triggers a biochemical cascade (The opening mechanism is activated).
- The ultimate outcome is a macroscopic response (The door opens).

The problem we examine here is how molecules can acquire specific messenger functions. Stated differently, how could Evolution endow molecules with biological meaning? The evolutionary explanation we propose is based on the facts that a) molecules exist within their property space; b) the molecular environment (e.g. a receptor) interacts with the molecules by constraining their property space (dissolvence) and so selecting some of their molecular states; c) in turn, the molecules act on the receptor by modifying it; d) this mutual adaptation creates a molecule-receptor complex, i.e., a complex system whose functional response is an emergent property; and e) this func-

tional response is a signal which becomes meaning in the higher levels of complexity — the biological context.

2 MOLECULAR STRUCTURE AND PROPERTY SPACE

2.1 THE CONCEPT OF MOLECULAR STRUCTURE

The description of molecules may be approached by considering form, function, and fluctuation (Testa and Kier 1991; Testa et al., 1997). Molecular form ("what a molecule is") can be equated with molecular geometry, namely atom connectivity (2D-structure) and more realistically the 3D-structure. Components of molecular form are called structural attributes. Molecular function ("what a molecule does") is interpretable from observations made during experiments and is expressed as measurable or computable properties. Structure and properties (i.e., form and function) influence each other and are indubitably intertwined. The third component in this approach is molecular dynamics, namely the fluctuation in form and function (Prigogine, 1978).

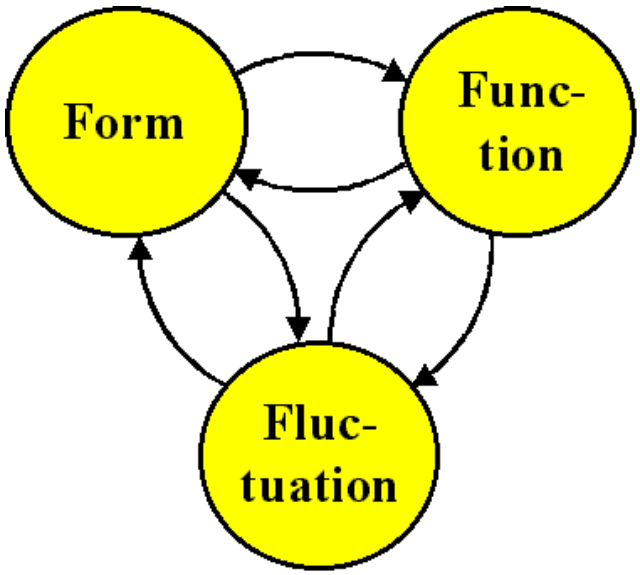

Figure 1: A comprehensive representation of molecular structure in the broadest sense, viewing form, function and fluctuation as its three essential components. A chemical compound can exist in a number of molecular states differing in conformation, surface area and volume, H-bonding capacity, polarity, lipophilicity, etc.

Form, function and fluctuation cannot be ordered causally or hierarchically. Rather, they are viewed as being of equal importance and feeding on each other, as schematized in Figure 1. Fluctuation influences form. To give an example, consider how in some compounds a labile hydrogen can jump from one position to another. This changes the atom connectivity (2D-geometry) of the molecule, which experiences a prototropic equilibrium and thus fluctuates between two or more states known

as tautomers. Similarly, the 3D-geometry of a molecule can vary markedly depending on its flexibility, resulting in stereoisomers separated by low-energy barriers and well known to chemists as conformers (conformational isomers). While tautomerism is restricted to a relatively limited number of compounds and involves two (seldom three) tautomeric states, conformational isomerism is a phenomenon of very frequent occurrence that produces a great many (an infinity depending on definition) conformational states. The ensemble of these states defines the conformational space, also known as the conformational hypersurface of a compound.

Form influences fluctuation. This is a rather trivial statement considering that the capacity of a molecule to oscillate between, e.g., tautomeric or conformational states is entirely pre-determined by its chemical constitution. This makes it clear that form and fluctuation are interdependent and influence each other, in complete similarity with the interdependence between form and function.

Function and fluctuation also influence each other. This is a conclusion that derives logically from the above statements, and which can easily be seen in chemical examples. That tautomers display different chemical properties is again well known to chemists. Similarly, it is common chemical knowledge that electronic properties (e.g. ionization state) will influence conformational behavior.

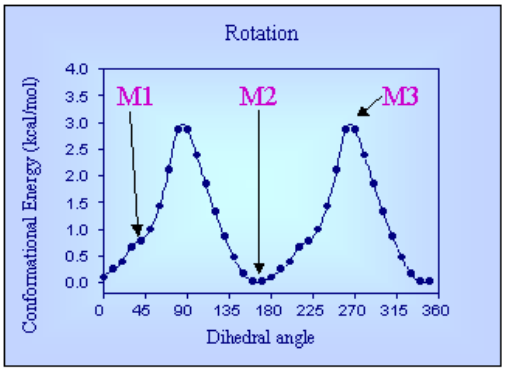

Figure 2a: The molecule of nicotine and its conformational energy when the dihedral angle between the two rings is rotated.

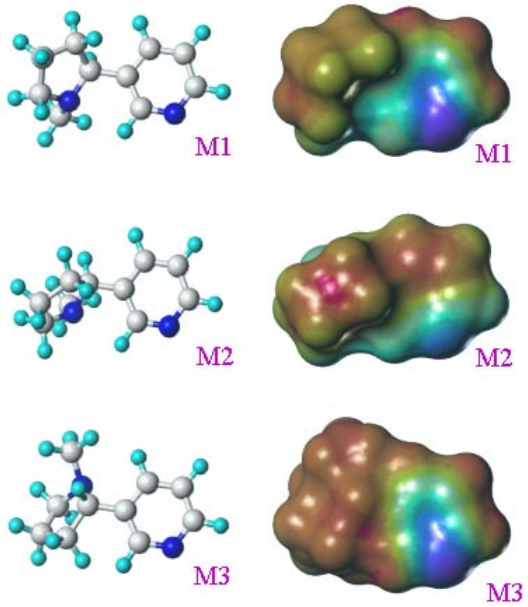

Figure 2b: Three representative conformers M1, M2 and M3 are shown. The form (geometry) of the molecule is seen to vary with the conformational state (left panels), as is its molecular electrostatic potential (right panels) taken as a representative property.

Nicotine is taken as an example here to illustrate the above principles. This compound has but one rotatable bond, and its conformational behavior is mainly described by the relative free energy associated with each value of the dihedral angle (Figure 2). Three individual conformers are shown in Figure 2, M2 being the most populated one since it corresponds to the global energy minimum, M3 corresponding to the rotation barrier, and M1 being of intermediate energy and probability. As mentioned above, the three conformers have clearly different forms (just consider the relative location of the two nitrogen atoms, but they also have different properties as exemplified by their different electrostatic potentials.

The general conclusion at this stage is therefore that form, function and fluctuation are interdependent. They depend on each other in a quantitative manner, and in some cases even qualitatively. This systemic and global perception of molecules has implications that will become evident below.

2.2 MOLECULAR STATES AND PROPERTY SPACE

Molecular fluctuation delineates the ensemble of all probabilistic changes a molecule can undergo in form and function. This generates a very large number of molecular states, which are snapshots of the molecule at a given moment in time. Each state is characterized by a unique combination of geometry (form) and associated properties (function). Reciprocally, any property exhibited by a compound will thus have a distinct value for each molecular state occupied by that compound.

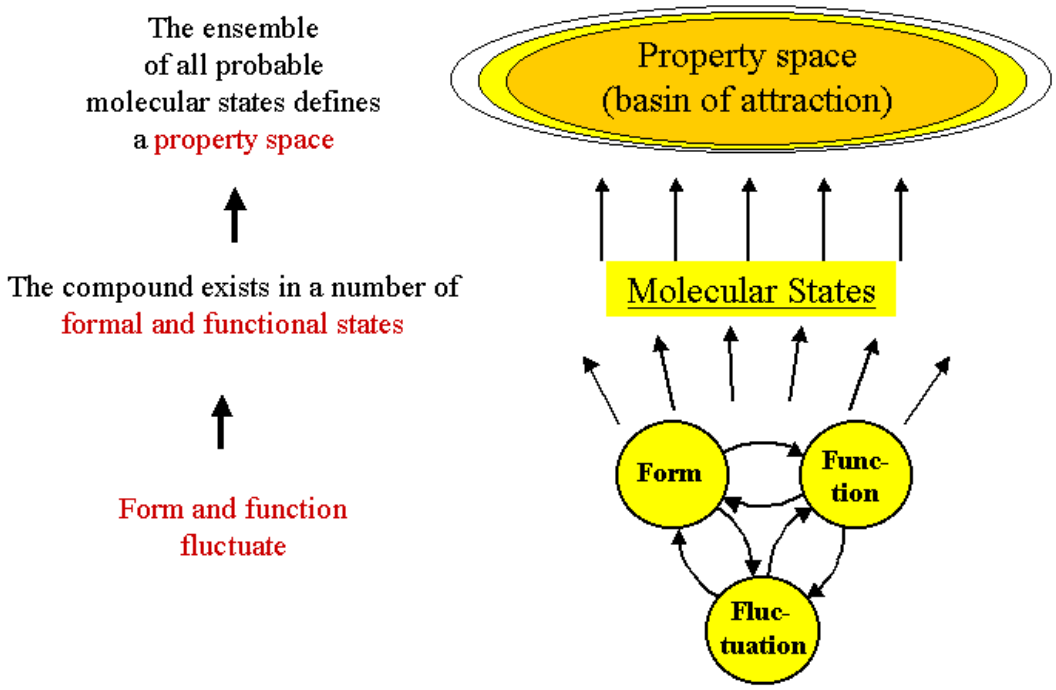

Figure 3: Molecular states are the expression of the mutual interdependence of form, function and fluctuation. The ensemble of all molecular states of a compound defines its property space, namely the range of values each property can span.

The ensemble of all possible states will span a range of values for all properties, thus delineating a property space. The latter can also be conceived as the basin of attraction of the property states of a compound. The concept of a basin of attraction can be schematized in pictorial language as shown in Figure 3. A physically more realistic representation of a basin of attraction of all molecular states is afforded by an energy landscape, namely a hypersurface whose dimensions are the energy of the system, plus all its other variables (Testa et al 1997). Usually, and this is the convention also adopted here, the more probable states of a molecule (i.e., its states of lowest energy) are represented as valleys in the energy landscape, whereas the states of highest energy are represented by peaks and the transition states as mountain passes. There is an energy maximum beyond which the molecule breaks down and ceases to exist, explaining why an energy landscape is finite. A schematic representation of an energy landscape will be shown later (see Figure 5 below). Note that any complex system could *a priori* be represented by an energy landscape, but the hyperdimensionality increases incommensurably for systems of higher complexity.

A molecular property of great biological relevance is lipophilicity, namely the preferential affinity of a solute for lipid-like over water-like solvents. This property is

commonly measured, but it can also be reliably computed from 2D– and 3D–structures (Carrupt et al 1997). In particular, an algorithm known as the Molecular Lipophilicity Potential (MLP) calculates a virtual lipophilicity for each conformer. The results have revealed large differences between the various conformational states of a compound, up to one order of magnitude or even more (Testa et al 1996). This phenomenon, which is particularly marked for large molecules such as various drugs and biomolecules containing both hydrophilic and hydrophobic groups (Jiang 1998), has been termed the chameleonic effect (Carrupt et al 1991).

It follows from the above that one way to rank all molecular states of a compound is along an axis of polarity, as will be done below in Figure 5.

3 MOLECULES IN A CHEMICAL CONTEXT: EXTERNAL CONSTRAINTS

3.1 THE MOLECULE–MEDIUM COMBINATION AS A COMPLEX SYSTEM

We now examine the interplay between a molecule and its molecular environment, showing that it creates a complex system in its own right. We note that a complex system results from interactions (also called transactions) between its components, and that it exhibits emergent properties non-existent in its components (Capra 1983). A third (and hitherto not explicited) feature of complex systems, that of dissolvence, will be discussed later.

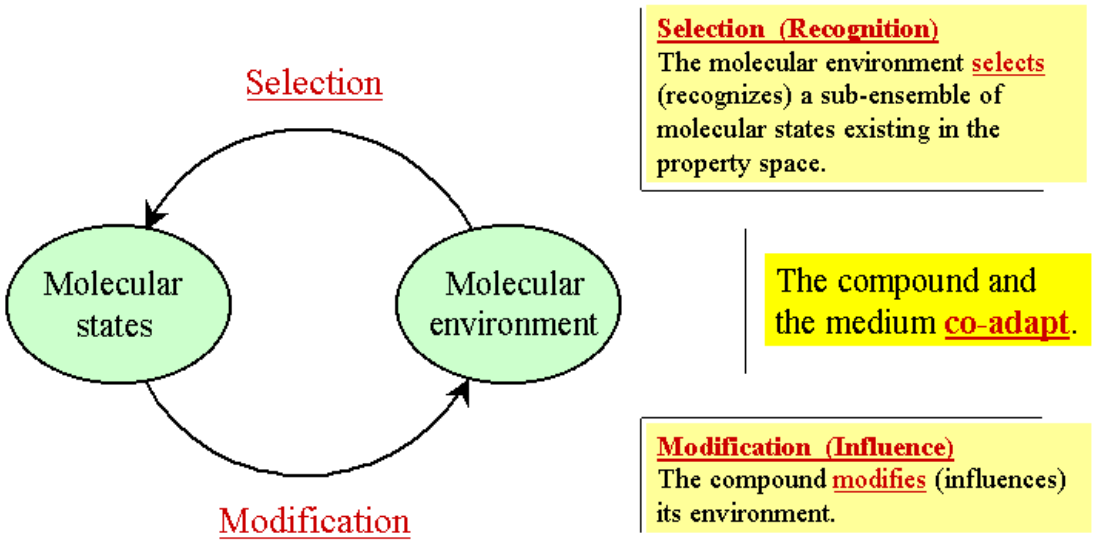

Figure 4: The complex system formed by a chemical compound and its environment results from two types of interactions between its components. The molecular environment selects a sub-ensemble of molecular states in the compound, whereas the latter modifies its environment.

As schematized in Figure 4, the molecule and its environment influence each other. At the macroscopic level, it can be stated that a compound modifies the medium with which it is in contact, as seen for example with changes in physical properties of a solution relative to the pure solvent, a modified fluidity in a membrane, or an allosteric effect in a protein.

But the medium also influences the compound. For example, a solvent will influence the electronic properties of the solute, which may exhibit changes in its color and UV spectrum. Similarly, the conformational behavior of the solute is markedly affected by the medium.

In the perspective of Figure 4, the molecule and its environment co-adapt to each other within their property space, a phenomenon that can also be viewed as a reversible co-evolution. In this writing, we focus essentially on the influence of the medium on the compound it engulfs. As shown below, this influence involves selection by the environment of a fraction of the property space accessible to the molecule. Such changes in property space have been termed dissolvence, being considered as the counterpart of emergence (Testa and Kier 2000).

3.2 SOLVENT CONSTRAINTS ON THE PROPERTY SPACE OF SOLUTES

Figure 4 not only schematizes the transactions between a molecule and its environment, it also raises the question of the intensity of their mutual adaptation. A precise

answer appears impossible, but the wealth of available experimental evidence ascertains a qualitative trend. Indeed, the degree of mutual adaptation between a compound and its environment depends mostly on the degree of organization of the latter. Here, we examine the case of a solvent, i.e., a medium with a low degree of organization. Biological media, which are characterized by a relatively high (membranes) or even an extremely highly (functional proteins) degree of organization will be discussed in Section 4.

Solvents have a rather high degree of macroscopic (apparent) order, but at the molecular level large random movements and fluctuations take place. The degree of mutual adaptation between solute and solvent will be comparatively low. The solute will have some influence on the solvent, e.g. by local alterations of its structure (e.g. the hydrophobic effect), and by altering slightly some colligative properties such as its freezing point, boiling point, vapor pressure and viscosity. As for the solvent, it usually has a marked influence on the properties of the solute. What is clearly revealed by experimental and computational investigations, for example, is the effect of the solvent on the conformational behavior of the solute, resulting in the selection of some among all the possible molecular states (Testa and Bojarski 2000; Testa et al 1999).

As a general rule, polar (hydrophilic) solvents will favor the more polar conformations and states, whereas the less polar (lipophilic) solvent will favor less polar conformations and states. In short, "Like selects like", a statement that extends the meaning of one of the most basic rules in chemistry, "*Similia similibus solvuntur*" (Like dissolves like).

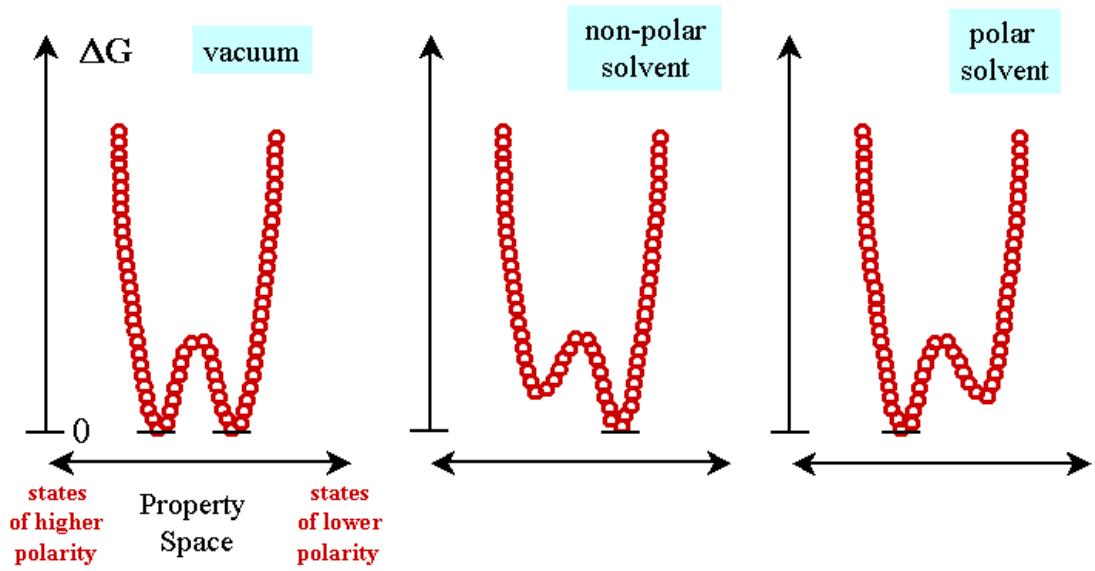

Figure 5: This figure shows the property space of a hypothetical compound as influenced by the molecular environment. Each recognizable state of the molecule is represented by a small cir-cle whose

position is defined by its relative energy (ordinate axis) and its relative polarity. In this representation, the property space is condensed to an axis of polarity, as explained in subsection 2.2. The compound is taken to have two low-energy states which are of equal energy/probability in the vacuum. In a solvent, however, "like selects like".

The experimental rule "Like selects like" can be depicted by Figure 5, which shows the property space of a hypothetical compound. Each recognizable molecular state is represented by a small circle whose position is defined by its relative energy (ordinate axis) and its relative polarity. In other words, the property space is reduced to and represented by an axis of polarity, as explained in subsection 2.2. The compound is seen to have two low-energy states of equal energy/probability in the vacuum. In a non-polar solvent, the solute will exist predominantly as conformers of lower polarity, whereas the opposite is true in polar solvents.

3.3 MUTUAL ADAPTATION AND THE EMERGENCE OF INFORMATION

As ambiguously encapsulated by Gel-Mann (1995), "Information is concerned with the selection from alternatives". This statement is also valid for the phenomenon described above, namely the recognition and selection by a solvent of some among all existing molecular states of a solute. But where does the necessary information originate from? Exclusively from the solvent, in which case all solutes would be identically constrained in a given solvent? Or exclusively from the solute, in which case the constraints experienced by a given solute would be solvent-independent?

Taken alone, none of these two explanations is satisfactory. In contrast, the information present in the separate components is mutually perceived when the chemical compound and the solvent encounter each other, interact and form the solute-solvent complex (the solution). This results in the emergence of new properties, in other words in the emergence of information.

The example of chemical reactivity can also serve to illustrate this phenomenon. Chemical reagents are seldom or poorly reactive as isolated molecules in the vacuum, but they need an adequate milieu to express their reactivity (i.e. to be "activated"). In fact, we are dealing here with a three-component system, since the reagent and its chemical target need an adequate milieu to enter the chemical reaction. There is also another deduction to be made from this example, that the property space of reagents is constrained toward increased reactivity. In this sense, it would be misleading to equate dissolvence with mere deprivation; rather, dissolvence implies constraints on (changes in) property space that cannot *a priori* be qualified as negative or positive. This however will not be discussed in the present writing. Suffice it to repeat that the informa-

tion necessary for a chemical reaction to proceed readily emerges in the reagent-milieu complex from information present in the components and upon their co-adaptation, as depicted schematically in Figure 6.

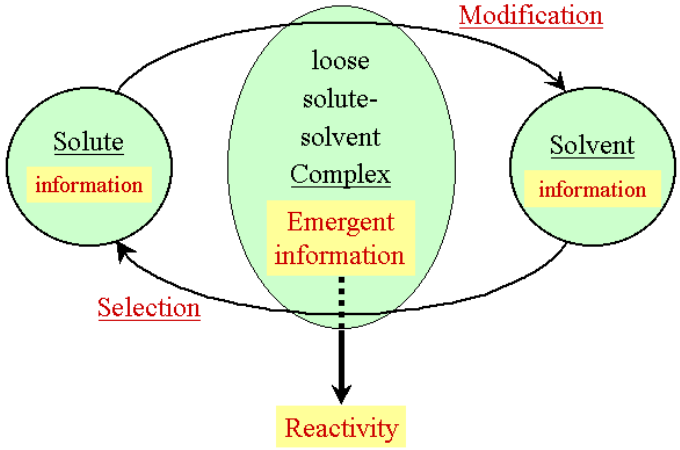

Figure 6: Scheme depicting the emergence of information upon formation of a solute-solvent complex system.

4 MOLECULES IN A BIOLOGICAL CONTEXT: THE EMERGENCE OF MEANING

4.1 MOLECULAR MACHINES

In section 3, we have seen how a non-biological (abiotic) medium with a relatively low degree of organization constrains a solute. We now turn our attention to biological media with an intermediate or high degree of organization. These are the media encountered by signal molecules such as hormones, neurotransmitters and drugs before and when they reach their sites of action.

Media with an intermediate degree of organization are for example biological membranes through which bioactive compounds must permeate to reach their targets. Here, we postulate an intermediate selection of molecular states and an intermediate degree of influence on the medium. Media with a high degree of organization are of great biological and pharmacological significance, being functional macromolecules such as receptors, nucleic acids, enzymes and transporters. The remainder of this text is restricted to the interaction of biomolecules with such highly organized biological media.

Functional biomacromolecules have a broad property space characteristic of their chemical integrity (i.e., their primary structure = the sequence of their monomers) which does not concern us here. But they have a second, very narrow property space with a rigidly defined tertiary structure (i.e., their 3D-structure) which contains the

single or the very few macromolecular state(s) compatible with functionality. Structural water (i.e., specifically bound water molecules) plays an essential role in stabilizing such specific states, not to mention a functional role in some enzymatic reactions (Kier and Testa 1996). Thus, fluctuations within the narrow property space of a functional macromolecule are compatible with its function, whereas fluctuations outside this narrow property space but inside the broader space destroy functionality while preserving chemical integrity.

The reason for such a remarkably high constraint on their 3D-structure is that macromolecules do not function in isolation but as constituents of functional assemblies some scientists like to call "molecular machines". These are complex systems of higher order resulting from transactions between functional macromolecules which recognize each other based on the stereoelectronic properties of exquisitely fine-tuned complementarity sites.

4.2 THE LIGAND–RECEPTOR COMPLEX AND ITS FUNCTIONAL RESPONSE

For the sake of simplicity, the following discussion will be restricted to receptors, bearing in mind — *mutatis mutandis* — that it also concerns other molecular machines.

Besides specific sites for macromolecule-macromolecule recognition, other high-affinity sites exist on receptors and other functional macromolecules to recognize and bind bioactive compounds. Here however, and in contrast to the loose solute-solvent interactions discussed above, strong transactions occur between the ligand (i.e., the bioactive compound), the macromolecule and structural water molecules. Such transactions are the mechanism by which a ligand-macromolecule complex is formed.

On the one hand, the ligand markedly influences the macromolecule by activating it (e.g. by an allosteric effect; Kenakin 1996) and triggering its biological response (in the case of an agonists or substrate), or by blocking its functioning (in the case of an antagonist or inhibitor). On the otehr hand, the influence of the receptor on the ligand is a remarkably strong one since a very narrow range of molecular states are selected, usually a single conformation. This is the well-known phenomenon of the induced fit (Koshland 1976). As long as the ligand-receptor complex lasts, the ligand remains practically frozen in a single conformational state, a strong constraint that allows for a tight complex and, we postulate, for an effective and selective biochemical response. Here like in solute-solvent systems, the information present in the separate compo-

nents is mutually perceived as the components recognize each other, co-adapt, and form the ligand-receptor system (Figure 7).

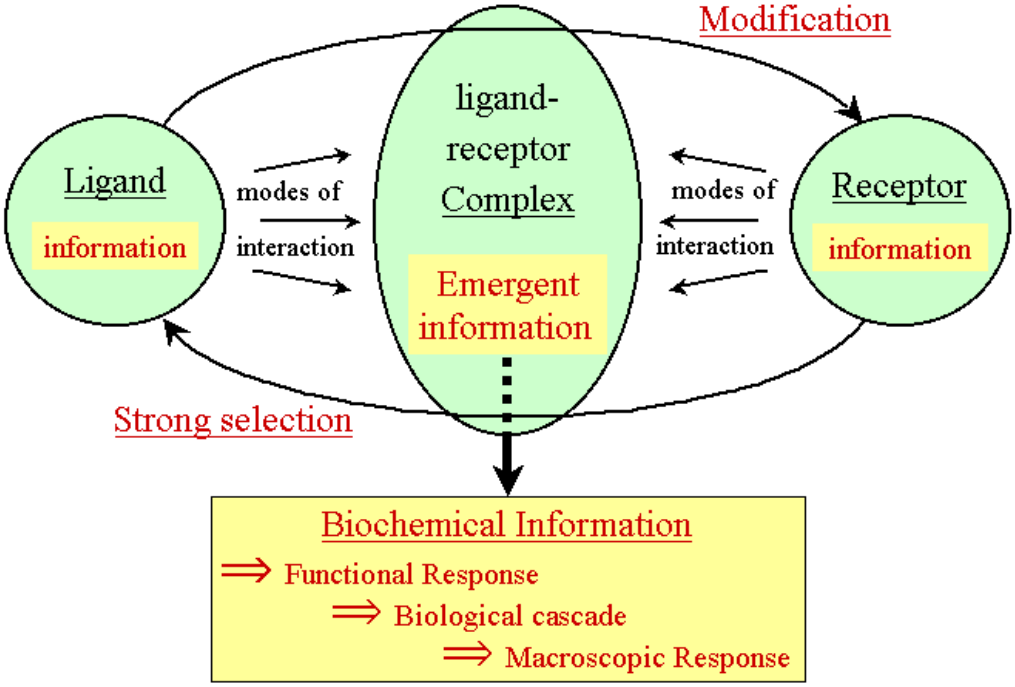

Figure 7: Scheme depicting the emergence of information upon formation of a functional ligand-receptor complex system. The activated receptor generates a biochemical response that will be amplified downstream into a macroscopic biological response.

In this co-adaptation, the receptor selects a restricted number of molecular states in the ligand, whereas the latter influences (modifies) the latter. Feedback control also exists in such a system, which thus becomes a model of the flow of information in a functional ligand-receptor complex. In such a simplified model, neither the ligand nor the receptor alone can produce a complete functional response, the functional response being indeed an emergent property of the ligand-receptor complex.

4.3 NESTED BIOLOGICAL CONTEXTS AND THE EMERGENCE OF MEANING

As discussed in the Preamble of this writing, a variety of bioactive compounds have a signal function. There is here an essential difference with abiotic systems since the emergent information can be translated into a functional biochemical reaction that in turn will cascade and be amplified into a macroscopic biological response. Figure 7 depicts the emergence of biochemical information, but it falls short of explaining how bioactive molecules can trigger macroscopic responses, in other words how they become endowed with macroscopic biological meaning.

In an enlightening text, Cohen and Stewart (1995) have explained and exemplified that no message exists that would contain inherent information independent of the context. One of their examples is particularly inspiring:

"When you transmit "Make me a pearl" to an oyster, the message takes the physical form of a tiny piece of grit but produces a wonderful, lustrous result. [.....] Meaning is a quality, not a quantity, and is highly dependent upon context."

How is this relevant to our discussion? When the piece of grit enters the oyster, it triggers a biochemical reaction in some cells and forces them to produce a solid secretion known as mother-of-pearl. However, the entire biological context, namely the oyster, is needed to produce a pearl from the mother-of-pearl, in other words to "inform" the mother-of-pearl. As made clear by Cohen and Stewart, if all the information needed to make a pearl were encoded in the tiny piece of grit, the latter should contain an amazing amount of it. In fact, the piece of grit only contains the information to interact with cells. The latter have the latent capacity to secrete mother-of-pearl, but they need a stimulus. Mutual recognition and interaction between grit and cells must occur for a functional response to be produced, in this example for mother-of-pearl to be secreted. However, only the oyster can give form to the mother-of-pearl and transform it into a pearl. Stated differently, the oyster is the biological context that gives meaning to the mother-of-pearl by creating a pearl.

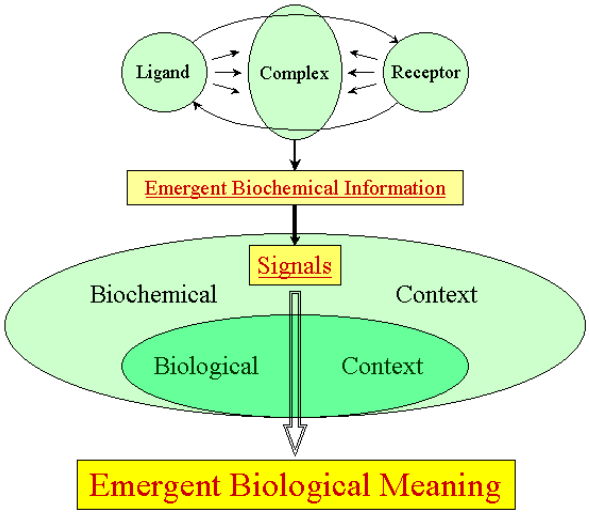

Figure 8: Scheme depicting the emergence of meaning resulting from the interaction between biochemical information (the functional response of the ligand-receptor complex) and a hier-archy of nested biological contexts.

Returning to bioactive compounds and their receptors, we can expand Figure 7 by stating that while biochemical information emerges in the ligand-receptor complex, it

needs higher levels of biological complexity to become macroscopically meaningful. These levels of higher complexity are the biological context, or better said a hierarchy of nested biological contexts (organelles, cells, tissues, organs, systems, organisms, ...). At each level, recognition and transactions must occur for the information to become a meaningful signal recognized by the next contextual level, and so on. The resulting spiral is depicted in Figure 9 and leads to the deduction that the autoregulation of organisms is based on a genuine bootstrapping of information and meaning.

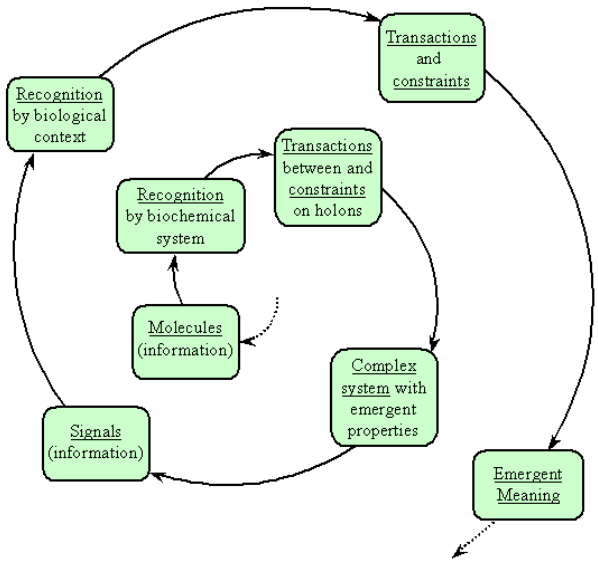

Figure 9: The bootstrapping of biological information and meaning, as deduced from the actualization of information occurring in nested biological contexts.

5 CONCLUSION: EMERGENCE AND DISSOLVENCE IN THE EVOLUTION AND AUTOREGULATION OF LIVING SYSTEMS

To summarize, we have seen that constraints on the property space of molecules and macromolecules (dissolvence) are inherent in biochemical recognition, which initiates the transactions between components (holons) that allow a complex system to be created (Figures 1–5).

The existence of such constraints reveals an information flow between the holons and the complex system. The information present in the holons allows their mutual recognition and the creation of the complex system. (Figure 6).

In the complex system, information emerges as a response, often in the form of a signal sent outward. Such signals will be recognized by and interact with the next level of biological complexity (Figures 7–8).

The spiral of dissolvence and emergence thus progresses in a hierarchy of nested biological contexts (Figure 9). Levels after levels of biological meaning are thus created by bootstrapping, propelling the evolution of living systems toward levels of ever increasing complexity (Figure 10).

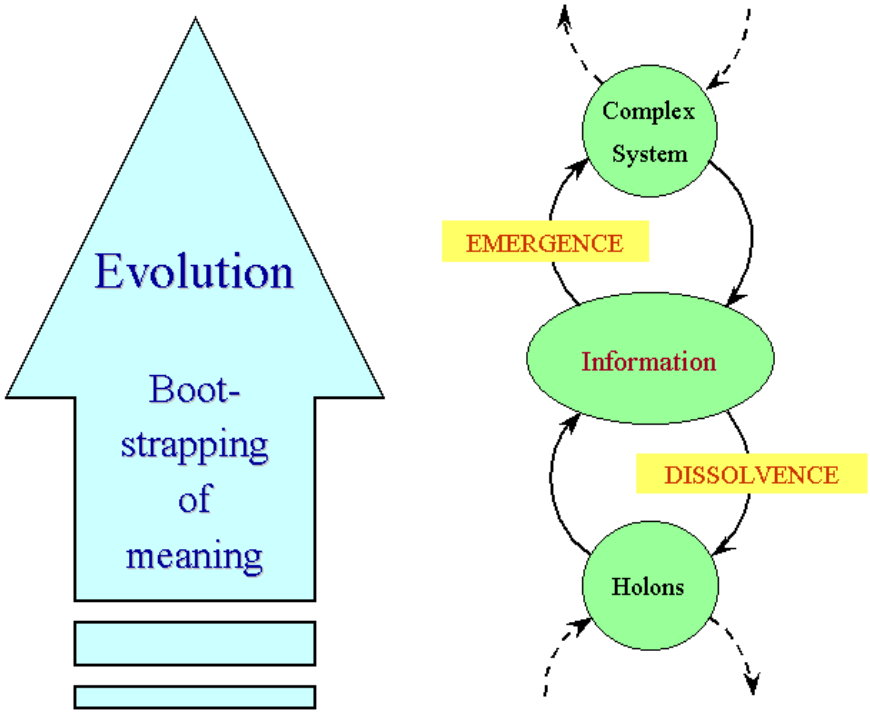

Figure 10: The coupled processes of dissolvence and emergence are the channels by which information is exchanged between holons and complex systems. Biological meaning emerges by boot-strapping, which propels the evolution of living systems toward ever increasing levels of complexity.

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