Challenging biomorphic sensing: the RECEPTRONICS project

Marco Tartagni

II. School of Engineering, University of Bologna, Via Venezia 52, 47023 Cesena, Italy

Advances in technology have pervaded our lives in the past fifty years, and even more significantly in the last ten years since the advent of the Information Society. Moreover, the ever-increasing perfection of technological products has made us accustomed to instruments that are increasingly more precise, affordable and secure. We should however note that we would not be able to talk to people around the world with a personal portable device that fits into our top pocket without the past efforts and achievements of hundreds of thousands of researchers working in the field, most of whom are unknown to the general public, and the rich multimedia experience that we enjoy in today's world of the internet and consumer electronics would not have been possible without the results gained by those teams.

Despite the huge complexity of devices, systems and protocols so far implemented, a closer look at Information Society technology shows that only a few concepts have been fruitfully exploited with regard to the huge variety of different tasks. One of the best known of these concepts is the one that information is transmitted by means of waves. The wave is one of the simplest representations in physics, merging concepts of circle and line together, and it is one of the simplest ways to represent the exchange of energy between physical systems. The wave is usually used for electromagnetic fields as an alternative representation to that of particles. Solar illumination, sound, earthquakes and sea waves are a few examples of how energy is commonly exchanged in our world.

Possibly belied by the technological advances announced daily, the true and inner driving force for the growing complexity of information systems is the ability of the researchers to come up with faster and more efficient systems to deliver and detect a large amount of information. Since *waves* are associated with *energy* (or *power*, taking into account time), and since *energy* is associated with *information*, the key aspect of modern technology is its capacity to detect or deliver information by energy as propagating waves. If the propagating signal is more complex than a single wave, we can treat it as being composed of a large number of "pure" waves of different *wavelengths* or *frequencies*. The collection of all the possible values of wavelengths is called *space* in our representation. The number of the possible values is called the *dimension* of the space. We can characterize any complex signal by splitting it into "pure" waves and looking at how their energies are distributed in space, that is its *spectrum*. Signal spectra are very important since they tell us how the signal energy (that is, information) is

Nanotechnology Perceptions **3** (2007) 35–42
Received 16 March 2007

distributed among the frequency components. This concept is very useful, since information (that is, energy) can be split among spectral components to increase data rate and efficiency. We see colours, hear sounds or transmit data over channels through this principle, where information energy is shared among elementary waves.

The question presents itself: how precisely can we detect a single signal among all the others? How far can we look with telescopes or how deeply with microscopes, since both are working on information-transmitting waves? How many telephone lines can we pack into a transmission channel, and how precisely can we detect radio signals in the free space of the universe?

With the concepts introduced so far, we could in principle sense indefinitely small signals or send an incredible amount of data over transmission channels. Since we know that this is not the case, something is missing in our framework. The problem arises from the fact that the universe as well as any physical system is pervaded by random signals superimposed on the information that we would like to detect. A surprising characteristic of those signals is that we cannot derive energy from them. This is a bit tricky: we did not say that those signals do not *have* energy, but that we cannot *derive* net energy from them. Those signals are called *noise*. Figure 1 illustrates the point with two examples.

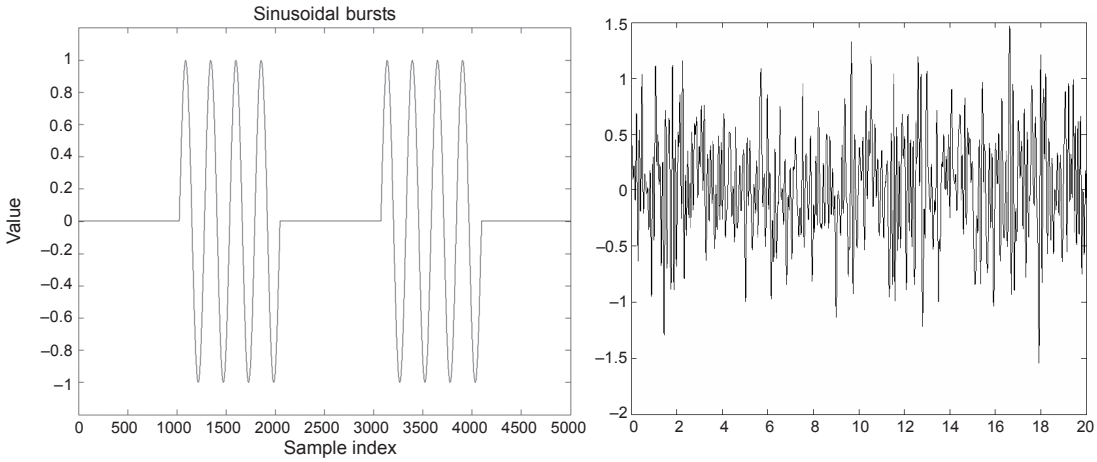

Figure 1. Left: a signal encoding information. "Sensing" means exploiting its intrinsic energy to detect such information. Right: an example of a noisy signal. Noise pervades any physical system. By being superimposed on the original signals they corrupt the information, lowering the detection capabilities of technology systems.

This is somehow not obvious, although it is a fundamental property of physical systems. Let's take an example. If we consider the motion of water molecules, we can always calculate the average kinetic energy of the molecules at a given temperature. However, due to the random motion of the molecules, we cannot extract energy from collections of their movements, unless a way of escape is found for them (i.e. through evaporation). This is stated in the second law of thermodynamics and nothing can be done to overcome that principle.

Turning back to waves, the same principle can be viewed as follows. Information could be detected by using the energy provided by the carrier itself. However, since physical processes are usually dominated by random phenomena, information energy is corrupted by the random

behaviour of noise. Thus, the main issue for any detection or communication system is to sense the energy of information signals over and above that of the noise. This is sometimes called the *signal-to-noise ratio*.

Communication and sensory systems *accuracy* is based on how we can deal with the signal-to-noise ratio. Very often noise energy is overwhelming with respect to the signal. Taking the example of Figure 2, where a noisy radar signal is displayed, at first sight the signal itself does not carry any information. The overall waveform is given by summing the two waveforms of Figure 1, i.e. the signal is corrupted by noise. Notwithstanding the noise, modern technologies can detect the original signal very precisely, as illustrated in Figure 2. How is it possible? The trick consists in using spectral considerations. If we know that the signal energy is distributed in a limited part of the spectrum whilst the noise energy is spread over a very wide band, we can use filters to select precisely that part in order to increase the signal-to-noise ratio. The approach is based on the idea of using *selectivity* to increase *accuracy*. In other words, selectivity and accuracy are an aspect of the same phenomenon, based on energetic balance. If the signal is relatively stable over time, we can wait to accumulate energy, gradually gaining over the widespread noise power. Using this approach, modern technology has achieved accuracies that are in some circumstances much higher than those provided by biological systems: electronic recording systems or CCD sensors can be hundreds or thousands of times more sensitive than mammalian eyes or ears.

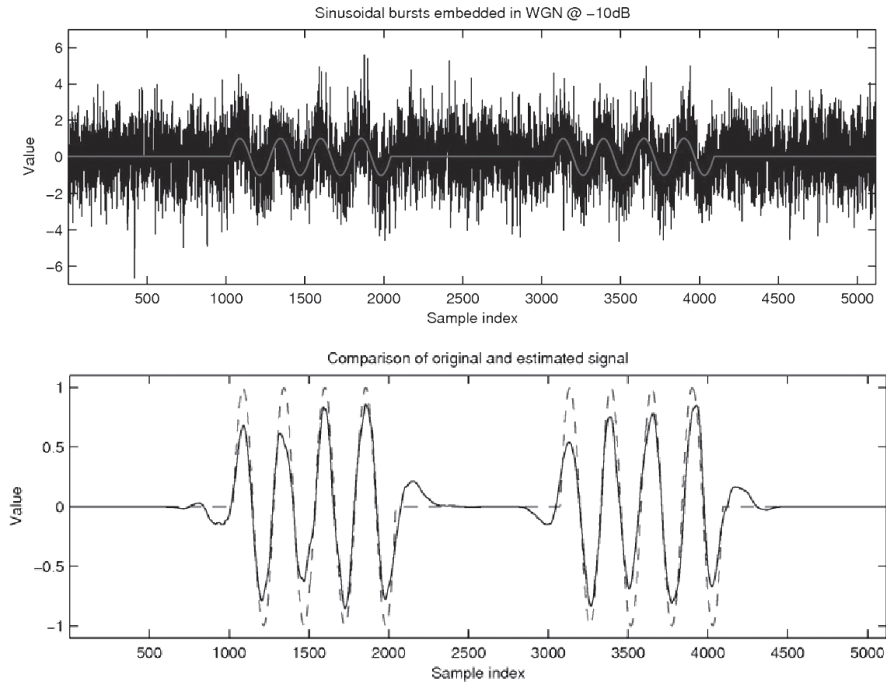

Figure 2. Above: a very noisy radar signal with superimposed burst signals carrying information. The noise energy is 100 times greater than that of the signal. Below: by exploiting spectral characteristic of signals and selectivity, modern data processing techniques can easily detect signals over noise (courtesy of N. Speciale, ARCES, Italy).

Chemical senses such as taste or olfaction are another story. For those senses, the mechanism of transduction is strictly related to the chemical properties of the sensed substances and not to the properties of waves, such as in seeing or hearing. They are based on molecular binding processes where technology still does not compete with nature. For humans the olfactory epithelium has an area of about 5 sq. cm, while for dogs it is 170 sq. cm and for cats, 20 sq. cm. To have an idea of these sensitivities we have to deal with molecule numbers. In a cubic cm (cc) of air there are about 2×10^{19} molecules. It is has been demonstrated that a dog's nose can sense 10 000 molecules of butyric acid per cc, which is a concentration of 5×10^{-10} parts per million. This is comparable to less than a hundredth of a drop of a volatile substance dispersed in a 100 sq. m room.

As far as technological achievements are concerned, state of the art electronic nose microsensors can reach sensitivities of the order of tens of parts per million. This means that the technological gap between vertebrate noses and today's electronic noses is about 10 orders of magnitude. If the epithelial area in dogs is very large, in some way accounting for such a high sensitivity, still more incredible data can be found regarding invertebrates. It has been estimated that moth sensilla can detect 1000 molecules of pheromone per cc for reproduction purposes. This demonstrates an increased sensitivity gap between nature and technology of eleven orders of magnitude: 100 000 000 000 times!

Why is there such a great divide?

Molecular binding techniques are essentially different from wave detection. It is the primordial process of biological communication, an extraordinary resource used by living organisms. A specific molecule maintains a three dimensional shape given by the structure of the bonds linking its constituent atoms to each other. This shape is as unique as the shape of a key. To recognise its unique features, nature builds another molecule whose shape is complementary in relation to the first one, as a lock is specific to its own key. The object nature uses to construct the newly shaped molecule is the most widely used building block of living organisms: proteins. They are long chains of a limited number of small molecules—monomers—whose sequence determines the form and the functionality of the final product. Living cells build them, while their structure and chemistry have been developed and fine-tuned over thousands of millions of years of evolutionary history. All proteins stick or *bind* to other molecules. The binding ability of constructed proteins can be strong or weak, though the binding always shows a great *specificity* or *affinity* in the sense that each protein molecule can bind just one or few molecules out of all the others, due to their complementarity. Some proteins are constructed to bind specifically to certain molecules in order to regulate biological processes. Others are specifically built to have an affinity to new molecules: this is how those proteins called antibodies attach to viruses or bacteria as a signal to the body's defence. Each antibody binds to a particular target molecule extremely tightly and thereby either inactivates the target directly or marks it for destruction. Usually the target molecule is called a *ligand* and the recognizing protein is called a *receptor*. Molecular recognition requires a detailed surface complementarity between a target molecule and its receptor. Figure 3 shows examples of receptors.

Even if the working principle is quite different from that relating to waves, it shares some common aspects. We have already shown the strong relationship between sensitivity and selectivity. In the molecular binding mechanism, the selectivity, now called *affinity*, is given by the peculiar way a complementary molecule attracts just one type among many others in a *space*.

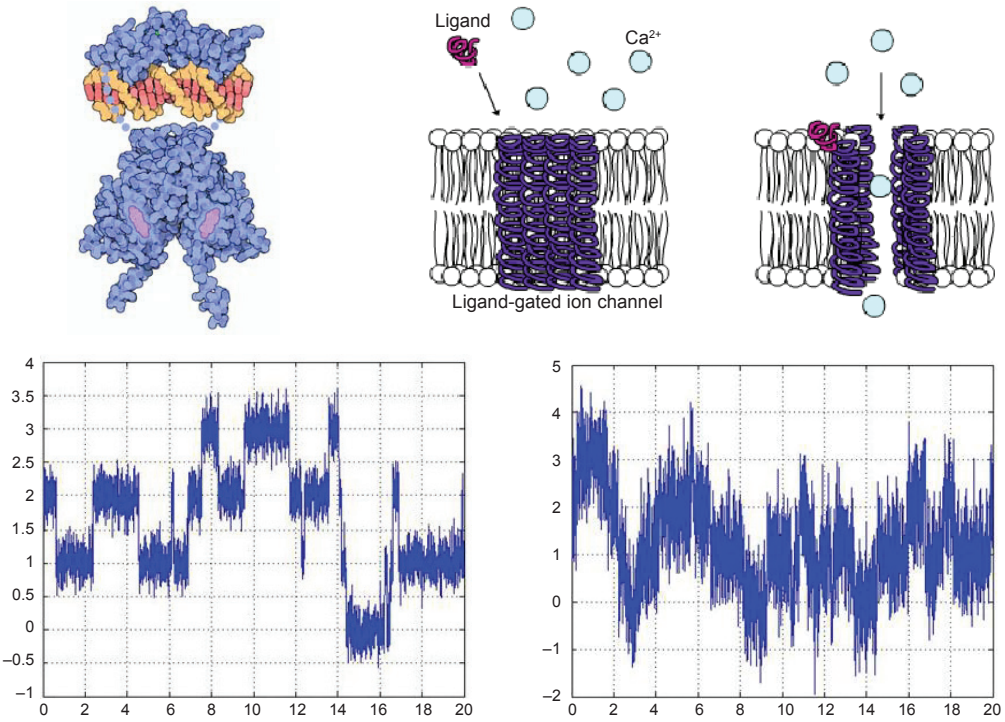

Figure 3. Top: the chemical interactions between an estrogen receptor and its ligand, a reactive molecule located in the nucleus of a cell. The two molecules form a complex structure that binds the DNA strands and promotes the transcription process of specific genes (© Protein Data Bank). Top, right: a ligand-gated ion channel. These are made from proteins placed across cell membranes, and are triggered by target molecules. When activated, they open a channel permitting ions to flow into and out of the membrane. Bottom: examples of signals (electrical current versus time) derived from transmembrane-gated ion channels, showing the ions in flux due to the activation of few biological macromolecules triggered by target molecules (courtesy of F. Lodesani, ARCES, Italy).

If waves can span certain selected bands of signals, chemical compounds can vary over an incredible number of different structures. To give an idea of the size of the numbers, here is an example: small molecules with at most a few dozen atoms play a fundamental role in organic chemistry and biology. It has been estimated that the size of the virtual space of small molecules that can be created from carbon, oxygen, nitrogen, and sulphur varies between 10^{18} and 10^{200}, with 10^{60} being the most reasonable value. On the other hand, the number of known small molecules encountered so far in nature or synthesized by man is of the order of 10^{7}. Clearly, by any of these estimates, chemical space remains rather unexplored.

The enormous sensitivity of nature's chemical sensors is once again based on a trade-off, as in the case of wave-based Information Technology: the greater the selectivity (affinity), the greater the accuracy (sensitivity). The big gap is due to the fact that modern technology does not have, at the moment, the instruments for effecting accurate molecular bindings. This is the reason why technology needs to borrow something from nature: the receptor paradigm. However, in doing so, new hurdles will have to be overcome.

Unlike most material objects, in which atoms are held together by strong covalent bonds, receptor and ligand bindings are made of weak non-covalent bonds. Non-covalent bonds are largely responsible for the secondary and higher order structure of biological macromolecules and are 1–3 orders of magnitude weaker than covalent bonds. For the above reason, molecular binding is not a *deterministic* but a *stochastic* event. For a ligand binding to a receptor, there will be a characteristic frequency with which existing ligand-receptor complexes dissociate as a result of thermal excitation, and a characteristic frequency with which empty receptors bind ligands forming new complexes, with a frequency of binding proportional to the concentration of the ligand in solution. Typical biological signals are illustrated in Figure 3. Here, a complex macromolecule is composed of a receptor linked to a channel embedded in the cell membrane. The channel is gated by a receptor with respect to a target molecule, allowing ions to flow inwards or outwards with respect to the cell interior. If we want to reproduce molecular sensing as nature does, we need to analyse as many events with regard to their individual interaction behaviour as possible. However, this poses new challenges to the task, due to the intrinsic stochastic behaviour of the binding events.

Molecular sensing is the primordial process of biological communication required by any regulatory process: cells send data by means of a complex network of molecular messengers. There are perhaps millions of regulatory substances in the human body and any imbalance between them may have dramatic consequences for well-being and health. Thus, molecular recognition is one of the first steps for a deep understanding of biological mechanisms, and being able to detect specific molecules at very low concentrations might be a promising new area for diagnostics. The availability of multiple biomarkers is believed to be especially important in the diagnosis of complex diseases like cancer. Patterns of multiple cancer markers might provide the information necessary for the robust diagnosis of disease in any person. Moreover, detection of markers associated with different stages of disease pathogenesis could further facilitate early detection. In fact, being able to detect specific biological molecules at very low concentrations is a new promising area of medicine that aims to identify the onset or prediction of disease before the patient shows any symptoms.

The need for reducing the sensitivity gap between nature and technology has recently led to several approaches. Nanowires, nanotubes, nanocantilevers and nanopores are examples aimed at attacking the problem for label-free molecular sensing, and very interesting preliminary results have been achieved. However, better results are expected by doing things as nature does.

Over the last 20 years there has been a sustained growth in interest of biomimetics as a problem-solving approach. What added value does biomimetics bring to the design process? How will this contribute to future adoption of biomimetics? In this context, the Receptronics project[1] is tackling the issue of molecular recognition. The project is funded by the European Commission and is being carried out by a team of nine institutions from all over Europe. Its name is given by the fusion of the words "re•cep•tor", and "e•lec•tron•ics" emphasizing the interdisciplinary research aimed at developing a label-free biomimetic platform for molecular recognition by merging both Information Technology and Biotechnology techniques. The approach is completely different from state-of-the-art electronic noses, where the technology is currently based on electrical conducting polymers—materials that are similar to plastics but can

[1] Item 4. on p. 42.

conduct electricity. These materials can be primed to absorb and respond to different odour molecules, and a typical artificial nose will feature an array of polymer sensors, each of which is responsive to a particular substance. However, odours affect many of the sensors in different ways, and the resulting pattern of responses needs to be analysed.

The Receptronics project has picked up the problem from a different angle with an emphasis on replicating what goes on in biology. In the natural nose there are cells with molecules embedded in the cell membrane. When these bind with an odour molecule, a cavity opens in the molecule and an electrical current flows, creating a stimulus, which is transmitted to the brain. The researchers will use molecular engineering techniques to create new artificial receptors, which are sensitive to different target molecules, as illustrated in Figure 4, left. These receptors will be embedded in membranes in an array, with each receptor linked to an electronic interface that can detect electronic signals transmitted when the receptor binds with its target molecule. The system will be mounted on a credit-card sized chip and a signal from each spot will be processed to collect data from their stochastic behaviour. The new nose is expected to have a sensitivity 100 to 1000 times greater than today's electronic noses and will be aimed, as a case study, at detecting hormones, representing a first step towards diagnostic applications. The goal of the project, therefore, is to combine the efficiency of the biomolecule with the powerful flexibility of integrated electronics in a unique device to be called a "Receptron". It is expected that the goal can only be achieved by using several strategies belonging to both Nanotechnology and Information Technology. This is the reason why Receptronics is organized in a stack of technologically objective layers, as illustrated in Figure 4, right, where each task is integrated and developed in a strong synergy with the others.

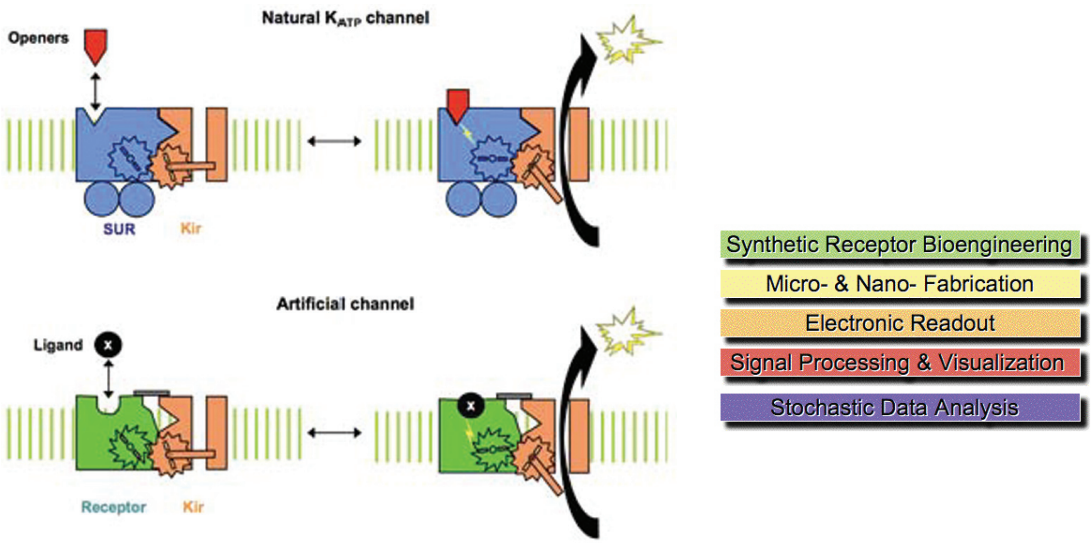

Figure 4. Left: bioengineered receptors tailored for specific molecules. Left above, a natural ligand-gated ion channel is activated by a target molecule. Left below, a new ion channel is bioengineered (a chimera) substituting the original receptor with another one, specific to a new molecule (courtesy of M. Vivaudou, CNRS, Fance). Right: Receptronics technology development requires a synergetic approach between disciplines.[1]

If the technology can be made cheap and simple enough for widespread use, it will enable the rapid identification and monitoring of proteins and pathogens. As a result, it will be possible not only to give appropriate medical treatment much more quickly, but also to make treatment patient-specific, leading to fewer side-effects and faster patient recovery. The Receptronics approach could provide a technological breakthrough for sophisticated diagnostic tools in the field of early cancer diagnosis and hormone balance monitoring. Furthermore, the same technology could be employed for detecting contaminants at very low levels of concentration, with applications in environmental safety concerning agriculture and industrial processes.

Further reading

1. H. Bayley and P.S. Cremer, "Stochastic sensors inspired by biology", *Nature*, vol. 413, pp. 226–230 (2001).
2. G. Zheng, F. Patolsky, Y. Cui, W.U. Wang and C.M. Lieber, "Multiplexed electrical detection of cancer markers with nanowire sensor arrays", *Nature Biotechnol*., vol. 23, pp. 1234–1301 (2005).
3. E. Stern, J.F. Klemic, D.A. Routenberg, P.N. Wyrembak, D.B. Turner-Evans, A.D. Hamilton, D.A. LaVan, T.M. Fahmy and M.A. Reed "Label-free immunodetection with CMOS-compatible semiconducting nanowires", *Nature*, vol. 445, pp. 519–522 (2007).
4. www.receptronics.org