A DARWINIAN PROCESS: THE MOLECULAR EVOLUTION OF ENZYMES

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ABSTRACT: New concepts as ambiguity, promiscuity and plasticity have allowed getting a deeper insight in the mechanism of the Darwinian process implied in the molecular evolution of enzymes. Directed evolution in the laboratory collapses the time scale for evolution from millions of years to months or even weeks due that the key processes: mutations, recombination, screening or selection are carefully controlled by the experimenter. In order to get a more meaningful vision a more enlarged scientific background is presented. The philosophical reflections focus on the increasing information in the evolution process, which goes beyond the dualism matter-spirit, and on the successive emergences. Finally some ethical and theological reflections are proposed.

KEY WORDS: darwinian process, enzyme promiscuity, molecular evolution, information, emergence, biotechnology, creation, intelligent design.

Un proceso darwiniano: evolución molecular de los enzimas

RESUMEN: Nuevos conceptos tales como ambigüedad, promiscuidad y plasticidad han permitido proponer una visión más profunda del proceso Darwiniano que se da en la evolución molecular de las enzimas. La evolución dirigida en el laboratorio recorta la escala temporal de la evolución de millones de años a meses o incluso semanas, debido a que los procesos claves: mutaciones, recombinación, ensayo individualizado o selección están perfectamente controlados por el experimentador. Para obtener una visión más rica se presenta un panorama científico más amplio. Las reflexiones filosóficas se centran en el aumento de información a través de la evolución, que supera el dualismo materia-espíritu, y en las sucesivas emergencias. Finalmente se proponen algunas reflexiones éticas y teológicas.

PALABRAS CLAVE: proceso darwiniano, promiscuidad de las enzimas, evolución molecular, información, emergencia, biotecnología, creación, diseño Inteligente.

1. Introduction

Molecular evolution emerged as a scientific field in the 1960's as researchers from molecular biology, evolutionary biology and population genetics sought to understand recent discoveries on the structure and function of nucleic acids and proteins. Some of the key topics that spurred development in the field have been the evolution of enzyme functions and the use of nucleic acid divergence as a «molecular clock» to study species divergence. Here I focus on the topic: molecular evolution of enzymes.

Enzymes speed up the rates of specific reactions, in such a way that they control the processes in living beings. They display enormous reaction rate enhancement in water at moderate pH values and mild pressures and temperatures, which correspond to the standard physiological conditions of live organisms. In fact, reactions with half times approaching the age of Earth are accelerated in many orders of magnitude up to 10^{20}, reaching time scales compatible with life. Examples

can be found in the survival of paper documents or ancient ships for long periods under water, which can be explained by the fact that the glycosidic bonds of cellulose are very resistant to hydrolysis in the absence of cellulases that catalyse their hydrolysis [1].

The enzymes are proteins, which are very complex macromolecules. From a structural point of view, four levels of complexity can be distinguished: primary, secondary, tertiary and quaternary structures. The primary structure describes the sequence in which amino acids have joined together to form the polypeptide. For instance, with twenty natural amino acids, in a small protein with 100 amino acids there are 20^{100} possibilities, which mean an astronomical amount. Only few sequences have been used by nature. Hydrogen bonds between different parts of the peptide chain backbone determine the secondary structure, α-helices and β-sheets being the most important three-dimensional conformations. Tertiary structure describes the way in which the secondary structure is packed to form regions of defined three-dimensional shape. Finally, these subunits may associate in a more complex system. It is the quaternary structure. The folding of a protein plays an important role in its functional behaviour. It must be emphasized that it is not straightforward to deduce the three dimensional structure of the protein from the knowledge of its sequence of amino acids. In fact, this goal has been only reached in proteins with a small number of amino acid residues. There are so many local minima in the energy conformation space that prevent to reach the global one.

Moreover, it would be even more difficult to relate the structure of the protein and the function. Optimizing the enzymatic function is a much more subtle problem, since mutations of residues in the active centre, that presumably could improve the efficiency of the enzyme, can decrease the stability of the full protein. Enzymes have evolved under selective pressure to both maintain the stability of the overall structure and the biochemical function. Two opposed trends, on one hand enzymes fold into compact structures; on the other hand they must also be active to catalyze chemical reactions. The active site of an enzyme is highly strained because is designed to develop favourable interactions with the transition state of the catalyzed reactions. This strain diminishes the stability of the global structure of the enzyme and thus a trade-off between stability and function can be established [2].

Enzymes must be of the adequate shape in order to exhibit specificity: they must be able to recognize and bind the correct substrate. However, an enzyme's structure must also be flexible in order to reach a complementary shape of a particular substrate and of its transformations. Furthermore, for an efficient catalysis the dynamic of the protein must be coupled to the dynamic of the reaction. Overall, they push the reactants to reactive conformations and stabilize preferentially the transition state [3]. One can to state that enzymes are more efficient machines than any machine designed by man. A feeling of admiration fulfils the researcher and, if he is a believer of a creator God, of adoration.

2. Enzyme evolvability

Evolvability, the capacity of evolutionary adaptability to external conditions, together with reproduction, characterizes the living beings. In this section, this feature of life will be stretched out to enzymes. A traditional view of enzymes holds that their catalytic activities, while optimized by evolution, also represent highly dead ends (one gene, one function). However, it has been recently suggested that this paradigm, that has dominated thinking in this field, could be too simplistic. In this sense, despite an enzyme is generally defined as a selective catalyst capable of differentiate between different substrates and speed up the rate of a particular chemical reaction, some enzymes have been found to present promiscuous activity, accepting alternative substrates and catalyzing secondary reactions [4]. This promiscuity provides a raw starting point for the evolution of enzymes, as a new duplicated gene presenting low activity would provide a start for adaptative evolution. In fact, new enzymatic functions can evolve in the period of years or even months, as happened recently with new synthetic chemicals or drugs [5].

Promiscuity may be classified as substrate promiscuity (ambiguity), the enzyme accepts structurally distinct substrates but catalyzes the same chemical reaction, or catalytic promiscuity, the enzyme accepts different substrates and catalyzes different overall reactions [6]. An example of the substrate promiscuity can be illustrated with the Cytochrome P450 (CYP), a vast family of enzymes found in almost all life forms. One of the most striking characteristics of some CYPs is that individual enzymes can interact with numerous structurally diverse substrates. This broad specificity usually serves by its obvious benefits playing an important role in metabolism. In fact, it is thought that more than 90% of drugs and chemicals oxidations in human are mediated by these promiscuous enzymes, probably based on the fact that they are not restricted to a particular substrate. These enzymes determine the bioavailability of drug molecules by converting them to more soluble, often inactive products that are readily excreted [7].

Another example of substrate promiscuity is the existence of antibiotic resistance mainly due to a family of enzymes called β-lactamases. The ancestral substrate of this enzyme was the penicillin but, after new generations of antibiotics are introduced these enzymes have co-evolved, broadening their activity to ever more elaborate antibiotics. β-lactamases hydrolyze β-lactam antibiotics' ring and thereby allow survival of pathogenic bacteria challenged by treatment with these agents. Metallo-β-lactamase (MBLs), which contain one or two Zinc ions bound in the active site, has become a severe clinical problem due to their especially broad substrate spectra and potential for horizontal transfers [8].

An example of catalytic promiscuity can be found with an enzyme that belongs to a new group of lactonases, the phosphotriesterase (PTE). It appeared on this planet only several decades ago from a pre-existing hydrolase with promiscuous activity to organophosphates. This system provides a powerful demonstration of the evolvability of enzymes. It has evolved for the purpose of degradation of synthetic insecticides introduced in the 20th century and may be considered as a

vestige of its progenitor which are likely to have existed for many millions of years. Interestingly, no naturally occurring substrate has been identified for PTE [9].

All these previous examples of promiscuity illustrate the enzyme evolvability, which means that enzymes are not dead ends but their efficiency can be enhanced or new catalytic activities derived from existing enzymes. Enzymes exhibit a remarkable evolutionary adaptability.

The enzymes favour, in a deterministic way, particular reactions inside a reaction network. A new function means an increase of catalytic space and an increase of complexity in the cell. It can be done in a chaotic form or in ordered one. As it has been frequently emphasized the life is at the edge of chaos. Nevertheless, what it is surprising in the self-organization of matter is to lead to stable systems able to reproduce in heritance.

3. Darwinian processes

Natural selection is a key factor in organism evolution, but the particular mechanism, by which it is carried out, is not well understood in the macroscopic world of phenotype. More information is available at the microscopic level of enzymes. It is widely accepted that many enzymes evolved from pre-existing enzymes via gene duplication. According to previous studies, promiscuous activity exhibit high plasticity as they can be readily increased by means of one or few mutations, allowing reaching the threshold for being improved under selective pressure. Instead, primary activity presents a large robustness against mutations, frequently they are neutral mutations. Divergent evolution requires duplication to free a gene from its previous functional constraints. Random drift will cause an accumulation of mutations in duplicated genes, however, many of which will be deleterious to structure and function, thus rendering the probability of obtaining a new function extremely low, even in evolutionary terms. If random drift has such low probability of generating a functional gene, how have enzymes evolved to catalyze such a remarkable diversity of reactions? Perhaps enzymes that evolved to catalyze one chemical transformation can, with some frequency, also catalyze alternative reactions at a low level. Such alternative activities might then provide the raw material for the evolution of new enzymes, as a newly duplicated gene that has an activity near the threshold level required to provide a selective advantage would have a head start towards being captured by adaptive evolution. Before this, there was only a latent function [4].

As previously mentioned, several contemporary enzymes catalyze alternative reactions distinct from their normal biological reactions. In some cases the alternative reaction is similar to a reaction that is efficiently catalyzed by an evolutionary related enzyme. Alternative activities could have played an important role in the diversification of enzymes by providing a duplicated gene a head start towards being captured by adaptive evolution. The alkaline phosphatase superfamily and the enolase superfamily will be presented as examples.

The catalytic promiscuity, the ability of an enzyme to catalyze, at low level, a reaction other than its cognate reaction that is maintained via selective pressure, provides a unique opportunity to dissect the origin of enzymatic rate enhancement via a comparative approach. The alkaline phosphatase (AP) superfamily is a perfect system to use in making such comparison. Thus, AP family, which catalyzes the hydrolysis of phosphate monoesters, presents promiscuous activities towards sulphate monoesters, phosphate diesters and phosphite [10]. Starting from this promiscuous activity, few mutations would be needed to reach a threshold value that could provide a selective advantage, allowing natural selection to optimize new enzymes in the superfamily. These observations explain the divergence of superfamilies from a common ancestor. This could be the case of nucleotide pyrophosphatase/phosphodiesterase (NPP), that present a highly common structural features with AP but preferentially hydrolyzing phosphate diester [11].

Conservation of structural and catalytic features in the enolase superfamily strongly suggests that enzymes of this superfamily arose via divergent evolution from a common ancestor to accept different substrates and catalyze different reactions. Divergent evolution of function in enolase superfamily presumably begins with duplication of the gene encoding a progenitor so that the original function can be retained as the mutations required for a new function to accumulate in the copy. The progenitor would catalyze a different chemical reaction, although it would employ the conserved catalytic strategy of using the Mg^{2+} cation to stabilize an enediolate intermediate. Important questions to answer include (a) whether the progenitor is promiscuous and already catalyzed the new function, (b) the number and location of mutations required for the new function to provide a selective advantage, and (c) the number and location of additional mutations required to optimize catalysis [12].

4. Protein engineering

Scientists have been always looking at Nature in order to find a source of inspiration to mimic the great power and efficiency of its processes. One of these astonish processes is the catalytic properties of the enzymes; very complex molecular machines capable of enhancing the rate constant of chemical reactions. One of their typical characteristic is evolvability. In fact, new enzymes or improved ones may be obtained, as it has been done through many millions of years by nature. It is important to point out that improving the efficiency of an enzyme does not necessary means to increase the rate constant of the catalytic process; it can be interesting to improve the robustness of the protein structure against a wider range of temperatures and solvents. This target could be useful for certain industrial purposes. The practical procedure to modify an existing enzyme is the field of protein engineering.

One day, we shall be able to *a priori* design amino acid sequences that will fold into proteins with desired functions. As this is not yet possible, scientists have

used *directed evolution* to generate molecules with novel properties starting from natural enzymes. Evolution, normally applied to animals and plants, requires the generation of variants and differential propagation of those with favourable features. Biologists and chemists have recently begun to use evolutionary strategies to tailor the properties of individual molecules instead of the whole organisms. Random mutations or recombination, can, in many cases, be done efficiently, leading in this way the molecular evolution in the laboratory. The successful variants can be identified either by screening or by selection. While screening requires an active search of all variants, selection is based on the exclusive survival of organisms containing the desired variants of the protein mimicking the true Darwinian evolution. This is an iterative process that requires, before starting new iterations of the process, the favourable variants to be amplified by clonation. The challenge is to collapse the time scale for evolution from millions of years to months or even weeks. Evolution does not work towards any particular direction, nor is there a goal; the underlying processes occur spontaneously during reproduction and survival. In contrast, the laboratory evolution experiments often have a defined goal, and the key processes (mutation, recombination, and screening or selection) are carefully controlled by the experimenter. The general techniques of directed evolution mimic natural evolution processes such as random mutagenesis and sexual recombination [13]. Thus, new proteins with new desired functions can be derived through mutations of few residues or recombining fragments swapped between two parent sequences. In this last technique it is possible to explore distant regions of sequence space, while this is not generally possible using random mutations [14]. In both cases these techniques allow to engineer enzymes without understanding them in great detail.

Another option is the *rational design* approach that consists in direct mutation of residues on selected specific positions of proteins [15]. The selection of residues to be mutated is deduced from X-ray diffraction structures of the complex between the protein and a stable molecule (an inhibitor, a transition state analogue, a Michaelis complex, an intermediate or a product). Moreover, information of the molecular mechanism of the chemical reaction is required. Mutation of few amino acids can render an important change in the active site of the enzyme, while structure of the full protein remains almost invariant. As a consequence, significant catalytic effects can be derived. However, the lack of knowledge on the relationship between amino acid sequence, protein structure and function, together with the extreme sensitivity of catalytic activity to seemingly modest structural perturbation, make redesign of an enzyme in the laboratory so difficult. In this strategy, a computational study of the catalytic function of the protein can provide the information needed to design successful mutations directed to a particular purpose [16, 17].

It is interesting to note that while *rational design* is usually focused on mutations close to the active site, *directed evolution* are based on random mutations that, most of the times, belong to regions of the protein far from the active site. So, both strategies can be satisfactorily combined to get an improved

function. Once a gene with a new function at low level has been obtained by *rational design*, it may be optimized by *directed evolution*.

The starting point in biological catalysts design not necessary must be a pre-existing enzyme. In particular, almost three decades ago, immune-globulin proteins have been used to produce Catalytic Antibodies [18]. Catalytic activity has also been introduced sometime in inert protein scaffolds [19].

5. Reflections on molecular evolution of enzymes

5.1. *At scientific level*

The first question to be addressed would be, what is the connexion between molecular evolution of enzymes and human evolution? Molecular evolution of enzymes is part of human evolution. In particular, it has been shown in this chapter that Cytochrome P450 evolution allows the elimination of new therapeutic drugs, mainly in human liver. Furthermore, in other cases evolution can take place in bacteria, affecting human life. β-lactamases evolution in pathogenic bacteria explains the existence of antibiotic resistance, a serious clinical problem. But the evolution of enzymes in bacteria has sometimes a beneficial impact, as it happens with the apparition of a new enzyme; the phosphotriesterase (PTE). Organophosphate triesters have been widely used as insecticides for the last 60 years. Although toxic to humans, bacteria have evolved ways of degrading these compounds so that they do not accumulate in the environment.

Evolution is a well established scientific theory. As Dobzhansky pointed out in 1973, «nothing in biology makes sense except in the light of evolution» [20]. Natural selection is the key factor proposed by Darwin to explain the complex organization and functionality of living beings [21]. Nevertheless, the mechanism through which this job is carried out it is not clear at all. There is a big gap between the microscopic mutations of genome and the macroscopic changes in the phenotype. Molecular evolution of enzymes allows getting a deeper insight in the mechanism through which mutations in genome can translate in new enzymes. The promiscuous activity of some enzymes catalyzing secondary reactions is the key concept. It has been suggested that this promiscuity provides a raw starting point for the evolution of enzymes, as a new duplicated gene presenting low activity would provide to start for adaptive evolution [4]. Some neutral mutations with respect to the primary activity may increase the secondary one, in such a way that the threshold for being improved under selective pressure is reached. This property is known as plasticity. A last remark, promiscuity refers exclusively to catalytic functions. Nevertheless, many enzymes have been found to moonlight, it means to serve additional functions that are generally not enzymatic, but rather structural or regulatory [22]. The active site of an enzyme represents only a small part o its surface. Thus, there is ample opportunity to use other parts of the protein for other functions, which may be optimized by a similar mechanism.

Emergence, the occurrence of unexpected characteristics in complex systems, and creativity are the surprising looks of evolution. Natural selection by a selective pressure explains, at least in part, this fact. Nevertheless, for some authors self-organization of matter to an increased complexity is also required [23, 24]. The profound power of self-organization in complex systems is not yet well understood. The self-organization may be a prerequisite or the precondition of evolvability itself, since it generates structures that can benefit from natural selection. The particular topology of the fitness landscape of a function precedes the evolution by natural selection of this function. Proteins are complex systems with a characteristic structural and dynamic behaviour, that scientists have started to understand. At present the connexion between amino acid sequence, the three dimensional structure and the function has started to be understood, in spite that it is not yet full clarified. That is why molecular evolution of enzymes may be a favourable ground to get a deeper insight on the role of self-organization and natural selection in evolution.

Directed evolution of proteins in laboratory allows testing many hypotheses on molecular evolution of enzymes. As it has been previously mentioned, the challenge is to collapse the time scale for evolution from millions of years to months or even weeks. This is achieved controlling carefully the key processes: mutations, recombination, screening and selection by the experimenter. Nevertheless, this is not the main goal of the present effort. The real purpose is to get new robust enzymes that allow catalyzing industrial processes leading to new products of commercial interest. Biotechnology is one of the main fields of the current technology and introduces ethical issues as it will be discussed in other section. Here the present spectacular achievements must be emphasized. Genomics and proteomics are deeply connected with each other at various levels. In this chapter a proteomic topic, deeply connected with a genomic one, has been developed. Both are technology-driven fields in exponential growth. Proteins are the most complex macromolecules; a cell is the next step in the scale of complexity. *Synthetic genomics* or *genome engineering*, from a fundamental alteration of gene content in existing microbes up to creation of rationally designed and fully synthetic life forms, is yet another addition to the postgenomic gallery. Franken cell is not a new concept, what is definitely new is its migration from the world of fantasy to the world of real. The first steps leading to this long-range goal has been given in two different directions. The first one may be defined by the genome reduction projects motivated by both academic and industrial interests with the most fundamental questions of life at heart. It is a top-down approach searching the minimal gene set needed for sustaining life in a defined environment [25]. The second is a bottom-up approach in order to obtain artificial cells, starting with the synthesis of various infrabiological systems, where the topic of the present chapter is included. The main challenge now is to encapsulate the components in a single cellular compartment and ensure that they will work in concert in a controlled manner [26, 27]. *Synthetic biology* is a new and very active field of research. One nice example is the synthesis of artemisinin, an efficient anti-malarial drug. It is a natural compound found in some plants in northern China, but it is

too expensive for large-scale use in the countries where it is needed most. To reduce the cost Keasling's group has used synthetic biology to engineer microorganisms to produce artemisinin from renewable resources [28].

As it has been mentioned before, in the cell a complex network of interactions and reactions goes on in an ordered way. This is only the starting point of life evolution, being the emergence of sensibility in advanced organisms and of intelligence in complex neuronal systems the main steps of this wonderful process.

5.2. *At philosophical level*

The evolutive functionality of enzymes in teleonomic order of organisms allows arising some philosophical reflections. Enzyme selection introduces information on the way in which dynamic systems may maintain its functional stability in a determined environment. Furthermore, it supplies also an insight on evolutive logic, following a blind Darwinian process. This logic will imply structural changes which will cause the emergence of sensibility. In this way the evolutive logic is building up an objective rationality which leads the emergence of a psychical, conscientious and spiritual world shown in superior animals including the human species, in such a way that evolution rationality may be understood. These emergences appear to be not radical new things but as new forms of being linked to new matter organization, in a continuous process.

Molecular evolution of enzymes, implying self-organization of matter and the emergence of new functions, arises two philosophical issues: the spirituality of matter and the problem of emergence itself. Enzymes recognize some specific molecules and catalyze its transformation. It means a lot of information in its structure and dynamics. As it has been mentioned before genomics and proteomics are deeply connected. The whole information in living beings is coded in a macromolecule, the ADN. This macromolecule plays a central role not only as material support of information, but also in its transmission and development in living beings. The concepts behind self-assembly have accustomed chemists to the idea that molecules can be programmed to interact and come together in very specific ways, and artificial replicating molecules have demonstrated the principles by which chemical information can be transmitted and amplified. Supramolecular chemistry has lead to the discovering of chemistry as an information science. As supramolecular chemists and Nobel laureate Jean-Marie Lehn stated «for me, chemistry has a most important contribution to make to the biggest question of all: how does self-organization arises and how does lead the Universe to generate an entity able to reflect on its own origin». Information is not a material reality but an immaterial one, going beyond the dualism matter-spirit. The information is a quite different thing than the material support. Nowadays, in a non dualistic conception of matter-spirit, causality can be seen as transmission of information. This vision of spiritual dimension of matter is a new version of what Pierre Teilhard de Chardin stated in a more poetic language in the spiritual power of matter [29].

Emergence itself is a serious philosophical problem. Through evolution new complex systems with new qualities appear and what is new must be explained. The reductionism denies the existence of radical emergence and what is new may be explained from properties of the components of the complex system. Holism looks at the new properties of the whole system, which can not be explained from the properties of parts. In this perspective there are problems to explain where the new features come from. To talk about potentiality of the new system in the old one or talk about its latent state before its apparition, may seem to be a game of words. In a more dynamical conception of being probably it is easier to find a more satisfactory philosophical explanation. Any way, the scientific method tries, by definition, to explain complex systems from more simple ones and, consequently it adopts a reductionism point of view. In molecular evolution of enzymes an emergence of new functions, catalysis of new reactions, is produced, but it cannot be asserted that this is a radical emergence. Darwinian Theory is a gradual one, with natural selection going on as an effect of selective pressure.

5.3. *At ethical and theological levels*

Gene modifications in order to get new enzymes are only examples of the present biotechnology. The projects of artificial life, of organisms modified genetically or of gene modifications in humans with therapeutic goals or other purposes are also biotechnology activities, which arise at present exciting ethical debates. As contribution to these debates I should like to do some personal reflections. First of all, when one perturbs a complex system, one is never sure of the results. It means that there is always some risk. Risk is part of the adventure of life and the amount of tolerable risk in human action is function of the conservative or progressive mentality of each person. Nevertheless, it must be remarked that in the present growth of human population the most conservative position may imply the most risky one. In a responsible attitude on the tolerable risk, the first ethical duty is to increase the basic research in order to minimise the surprises.

In the perspective of evolution theory, as the one adopted in the present paper, the vision of a fixed nature is not compatible. The man as the arrows of the evolution, in Hefner's expression «created co-creator» [30], has the responsibility to lead the process. Our task is to make God present in the world, by transforming it. This engagement is not playing God but properly playing human. To see as sacred every thing belongs to an ancient attitude far away from the present scientific and technical one. Looking to gene modifications of the man genome, a special responsible attitude is required, but if this patrimony for the following generation may be improved, there is a duty to do it. Finally, if one is not an expert in biotechnology problems, there is a prudent position to be careful taking part in the ethical debate.

Creativity of life, of nature or of man, as pointed out in this chapter on the molecular evolution of enzymes, suggests a central theological topic, a creator

God. When people criticise creationism or intelligent design refer to literal interpretation of Genesis text, known as young earth creationism, or a particular theory of intelligent design, ID, which is at fashion in States the last years [31]. Believers do not question the creation of cosmos by God and his wonderful design of the world pointing out to man apparition. As stated in the introduction, the admiration of the researches for the rich complexity of nature becomes adoration in the heart of the believer. In particular, Christian doctrine has been rethought for the better in the light of Darwin. He has helped us to recover the sense that God's creative work is continuous. In Darwin's time there were many deists who took the heretical view that, though God has created things once for all at the beginning, he was no longer actively involved in creation. Darwin helped people to understand that creation is an ongoing project for God. Evolution is a remarkable way for God to have brought species into being.

Ayala asserts that Intelligent Design theory, ID, is a bad science and a bad theology [32]. The evolution is accepted to explain the origin of different species; nevertheless there are irreducible complexities like: eye complexity, bacterial flagellum, blood coagulation or immune system, which can not be explained by evolution and require the direct intervention of an intelligent designer. These irreducible complexities have not been proved and so, ID has no solid basis at the scientific level. The Darwin's fundamental discovery is that there is a process that is creative although not conscious. It is a creative process because it causes favourable mutations to combine and accumulate, yielding a great diversity of organisms over eons of time. There is a design without designer. The existence of mistakes, suffering, cruelty and sadism in evolution is consequence of the nature of this process, and must not be assigned to God, as it happens in Intelligent Design. For this reason Ayala thinks that this theory is also a bad theology [32]. Science and religion are at different levels and between them it may not be contradiction. Questions on the meaning and purpose of the universe and life, on the relationship between humans and their Creator or on moral values can not be answered by science. On the other hand, it is not the job of the religion to supply scientific explanations of natural phenomena.

References

[1] R. Wolfendendo and M. J. Snider, *Acc. Chem. Res*. *34* (2001) 938-945.
[2] C. Cheng, B. Qian, R. Samudrala and D. Baker, *Nucleic Acids. Res*. *33* (2005) 5861-5867.
[3] M. Roca, S. Martí, J. Andrés, V. Moliner, E. Silla, I. Tuñón and J. Bertrán, *Chem. Soc. Rev*. *33* (2004) 98-107.
[4] P. J. O'Brien and D. Herschlag, *Chem. & Biol*., 1999, R91-R105.
[5] O. Khersonsky, C. Roodveldt and D. S. Tawfik, *Curr. Opinion Chem. Biol*. *10* (2006) 498-508.
[6] M. D. Toscano, K. J. Woycechowsky and D. Hilvert, *Angew. Chem. Int. Ed*. *46* (2007) 3212-3236.

[7] J. D. Maréchal and M. J. Sutcliffe, *Curr. Topics Med. Chem. 6* (2006) 1619-1626.
[8] M. W. Crowder, J. Spencer and A. J. Vila, *Acc. Chem. Res. 39* (2005) 721-728.
[9] L. Afriat, C. Roodveldt, G. Manco and D. S. Tawfik, *Biochemistry 45* (2006) 13677-13685.
[10] I. Catrina, P. J. O'Brien, J. Purcell, I. Nikolic-Hughes, J. G. Zalatan, A. C. Hengge and D. Herschlag, *J. Am. Chem. Soc. 129* (2007) 5760-5765.
[11] J. G. Zalatan, T. D. Fenn, A. T. Brunger and D. Herschlag, *Biochemistry 45* (2006) 9788-9803.
[12] J. E. Vick and J. A. Gerlt, *Biochemistry 46* (2007) 14589-14597.
[13] F. H. Arnold, *Acc. Chem. Res. 31* (1998) 125-131.
[14] M. N. Carbone and F. H. Arnold, *Curr. Opinion Struct. Biol. 17* (2007) 454-459.
[15] K. L. Morley and R. J. Kazlauskas, *Trends in Biotechnology 23* (2005) 231-237.
[16] S. Marti, J. Andrés, V. Moliner, E. Silla, I. Tuñón and J. Bertrán, *Angew. Chem. Int. Ed. 46* (2007) 286-290.
[17] S. Marti, J. Andrés, V. Moliner, E. Silla, I. Tuñón and J. Bertrán, *J. Am. Chem. Soc. 130* (2008) 2894-2895.
[18] E. Keinan (ed.), *Catalytic Antibodies*, Wiley-VCH Verlag, Weinheim, 2005.
[19] M. A. Dwyer, L. L. Looger and H. W. Hellinga, *Science 304* (2004) 1967-1971.
[20] T. Dobzhansky, *Am. Biol. Teach. 35* (1973) 125-129.
[21] C. Darwin, *On the origin of species by means of natural selection*, John Murray, London, 1859.
[22] S. D. Copley, *Curr. Opinion Chem. Biol. 7* (2003) 265-272.
[23] S. A. Kauffman, *The origins of order: self-organization and selection in evolution*, Oxford Univ. Press, Oxford, 1993.
[24] S. A. Kauffman, *At home in the universe*, Oxford Univ. Press, Oxford, 1995.
[25] T. Feher, B. Papp, C. Pal and G. Posfai, *Chem. Rev. 107* (2007) 3498-3513.
[26] A. Pohorille and D. Deamer, *Trends in Biotechnology 20* (2002) 123-128.
[27] E. E. Szathmary, M. Santos and Ch. Fernando, *Top Curr. Chem. 259* (2005) 167-211.
[28] J. Kirby, D. W. Romanini, E. M. Paradise and J. D. Keasling, *FEBS Journal 275* (2008) 1852-1859.
[29] P. Teilhard, *Hymne de l'universe*, Éditions du Seuil, Paris, 1961.
[30] P. Hefner, in *Cosmos as creation: Theology and Science in consonance,* ed. Ted Peters, Nashville, Abingdon, 1989, p. 227.
[31] F. S. Collins, *¿Cómo habla Dios?*, Ediciones Temas de Hoy, S.A., Madrid, 2007.
[32] F. J. Ayala, *Darwin y el diseño inteligente*, Alianza Editorial, Madrid, 2007.

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