Have Scientists Already Been Able to Surpass the Capabilities of Evolution?

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Abstract

Over two millennia ago Socrates was pondering whether our Universe and all things in it are governed by randomness or by a regulating intelligence. This philosophical question has been alive till the present day, since the proponents of neither side have been able to convince their opponents. Scientists seldom express or recognize clearly their philosophical presuppositions and many think that there is no room for philosophy in science. Our view is that although science cannot determine which philosophical view is correct, it can show which one is wrong. Here we critically review the experimental results obtained during the past twenty years by Jack W. Szostak and his co-workers relating to functional information among random RNA and protein sequences. We explain in detail why their experiments with random or partially randomized protein sequences do not mimic the processes that take place in natural populations. Simple calculations show that in the laboratory scientists have searched much larger sequence space than could have been searched by random natural processes. We further argue that the discovery of singletons and of protein-protein-interaction networks has removed the randomness concept from biochemistry, and that neo-Darwinian view of the living world is false. We see faulty Hegelian logic as a major reason for the survival of the illusion that evolution is true, and the same logic is misleading many scientists into accepting empty phrases like "intrinsically disordered proteins" as existentially meaningful.

Introduction

The question posed in the title will sound paradoxical, puzzling or simply silly to some readers. They will think: if scientists like all other living organisms are the product of evolution, how could they have exceeded its power today when we are unable to produce a single truly new living organism? Having passed but a short distance on the way to creating such a new live organism, how can it be true that scientists have already reached beyond what evolution is able to do? And if a group of scientists has actually achieved this, how come in their publications they have not claimed credit for such a great achievement? The goal of this paper is to show that the answer to the title question is yes.

First let us define and clarify some terms. By *design* in a broader sense we mean "the way something is made so that it works in a certain way or has a certain appearance" (Macmillan Dictionary) and in a narrower sense "the arrangement of the features of an artefact, as produced from following a plan or drawing" (Oxford Dictionary). We also accept that *design* means "deliberate purposive planning" (Merriam-Webster Dictionary). By *evolution* we mean "a theory that the various types of animals and plants have their origin in other preexisting types and that the distinguishable differences are due to modifications in successive generations" (Merriam-Webster Dictionary), where – in line with the neo-Darwinian synthesis – the *modifications* refer to changes in DNA due to mutations and recombination. The changes in DNA occur at random and are fixed in successive generations by natural selection and/or genetic drift, the processes studied by population genetics. Here the term *scientists* has a narrow meaning to denote a group of people who are committed to discovery and defense of scientific truths, where the *scientific* means verifiable by experiment in the laboratory, by mathematics and/or logic. The *truth* means correspondence between what is claimed about some thing and what that thing is. A *philosopher* is a person seeking after and loving truth.

Having the experience of senior experimental biochemists, we know that a dispute among sci-

entists about a particular experimental result usually takes a short time to resolve when both sides have access to the same instruments and starting materials. When the instruments after several repetitions in two or more labs give the same result, the issue is settled. The instruments thus play a role of an arbiter, and today scientists are accustomed to accept their verdict without complaint. (We acknowledge, however, that in some cases the system studied is so complicated that it leaves various interpretations open; such is the case for example on many issues related to human nutrition, medicine or climate change.)

No such arbiter, however, exists to pass verdicts in philosophical disputes among scientists. Now, some readers might argue that there are no philosophical issues for scientists to disagree about, or that they are unimportant, or that scientists are unqualified for such discussions. We are of a different opinion. But instead of elaborating further on this point here, we prefer to go straight to the philosophical issues pertinent to the topic of this paper.

In Philebus of Plato (429–347 B.C.E.), Socrates asks a key philosophical question:

"Whether we are to affirm that all existing things, and this fair scene which we call the Universe, are governed by the influence of the irrational, the random, and the mere chance; or, on the contrary, as our predecessors affirmed, are kept in their course by the control of mind and a certain wonderful regulating intelligence."

During the past 2,400 years, philosophers have preferred either the first or the second possible answer, and the situation has not changed in our times. Since the two answers are contrary, both cannot be true. For those people who hold one answer as true, it is unthinkable that the other answer might be true. Therefore, it is not surprising that scientists who are in agreement about the truth on so many other things start to disagree sharply at the philosophical level, the level which they reach by generalizations from scientific data, or from which the scientists justify their starting assumptions when planning and conducting research. We wish to make our philosophical position on the above question clear: a regulating intelligence is needed to explain all existing things.

Many renowned scientists have shared and do share our philosophical position, many equally renowned scientists are against it, and many are either undecided or unwilling to state their philosophical position in public. While respecting all the choices, our highest appreciation is reserved for those scientists who have decided to publish their philosophical assumptions. We especially value the opinions contrary to ours, because the arguing against a philosophical position forces us to re-examine and clarify our starting assumptions as well as our conclusions.

In this paper we will critically review the work of Jack W. Szostak (Nobel laureate, 2009) and his co-workers related to evolution of proteins with new functions. Their work is of exceptional importance not only because of its methodological novelty, conceptual originality, depth and breadth, but also because of the clarity with which the authors express their philosophical assumptions and conclusions. Thus, in a paper published in 2007 [1], we can read:

"Life, with its novel collective behaviors at the scale of molecules, genes, cells, and organisms, is the quintessential emergent complex system. Furthermore, the ancient transition from a geochemical world to a living planet may be modeled as a sequence of emergent events, each of which increased the chemical complexity of the prebiotic world."

And in another paper from 2012 [2]:

"Simple chemistry in diverse environments on the early earth led to the emergence of ever more complex chemistry and ultimately to the synthesis of the critical biological building blocks. At some point, the assembly of these materials into primitive cells enabled the emergence of Darwinian evolutionary behavior, followed by the gradual evolution of more complex life forms leading to modern life."

It is evident that the philosophical position expressed by these authors corresponds, contrary to ours, to the first of the two alternatives proposed by Socrates. Here we will not comment on the presumed assembly of biological building blocks into primitive cells; instead we will focus on the alleged adequacy of evolution to generate from some primitive cells all life forms in existence today. We will inform the readers where we agree with the authors, where we disagree, and finally why and how we have arrived at conclusions opposite to theirs.

Functionality among random sequences

Functional information of proteins and RNAs

There is a general agreement among scientists that the sequence of building blocks of a biopolymer represents a type of molecularly coded information; it is the specific ordering of the nucleotides or amino acids building up DNA, RNA or protein molecules that determine their structure and function [3]. Proteins are the most versatile and efficient in terms of function; to be convinced of this it is sufficient to take just a glance at the poster with metabolic pathways showing the plethora of reactions catalyzed by enzymes [4].

The building blocks of the proteins that are present in all living organisms, from bacteria to humans, are 20 amino acids. The average number of these amino acids that build up a protein is about 300, more precisely, 267 for bacterial and 361 for eukaryotic proteins [5]. These 300 amino acids can be ordered in 20^{300} (10^{390}) different ways. Scientists generally agree, based on several lines of experimental data, that more than one specific protein sequence is capable of performing a particular function.

But scientists still debate on the size of the fraction of functional protein molecules among non-functional ones, as well as on how to best describe the functional information residing in proteins. The difficulty is confounded by experimental findings showing that there are protein families with over 100,000 members having related but different sequences and, most likely, essentially the same structure and function. Moreover, known are also proteins having different sequences and structures, but similar functions. How can one address this difficult issue?

A useful concept for evaluating functional information of proteins was described by Szostak and co-workers [1, 3], where the functional information correlates with the fraction of all possible sequences that achieve a specified degree of function. Hence functional information is not a property of any one molecule, but of a group of all possible sequences, which can be ranked by activity as schematically shown in Figure 1. Functional information increases when fewer and fewer sequences display the activity in question.

Formulating information mathematically

The functional information needed to specify that activity is $-\log_2$ of the fraction of the sequences displaying a certain activity. When functional information [I(E_x)] is calculated with reference to a specific degree of function x, designated E_x, existing in a system with N possible configurations of which M(E_x) achieve or exceed the specific degree of function, we have [1]:

$$[I(E_x)] = -\log_2 [M(E_x)/N]$$

The maximal possible functional information is obtained when just a single protein sequence exhibits a specified activity, Emax:

$$[I(Emax)] = -\log_2 [1/N] = \log_2 N$$

For example, if just one protein sequence 300 amino acids long exhibits a specified activity – which is a limiting and unlikely case – then the quantity of functional information is:

$$[I(Emax)] = \log_2 10^{390} = 1295.6 \text{ bits}$$

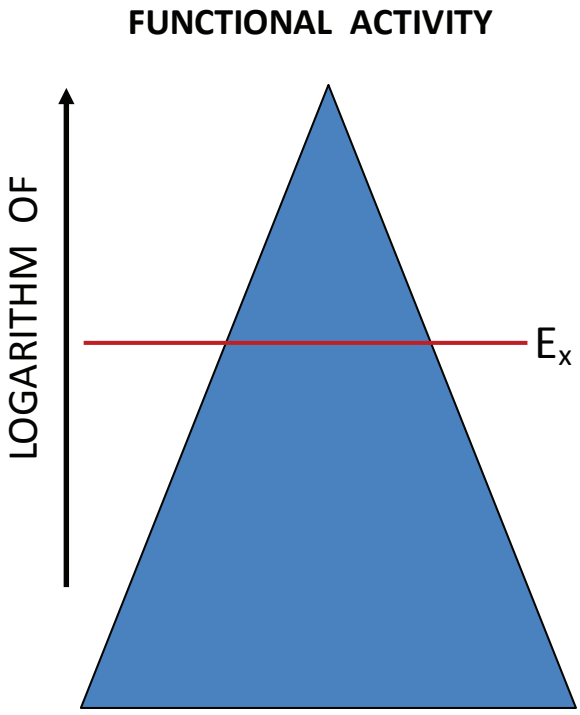

Figure 1
A schematic representation of functional information content that is present among biopolymer molecules of all possible sequences, for example RNA or protein molecules. A vast majority of the molecules is devoid of a particular functional activity, whereas a much smaller fraction possesses that particular activity. From that fraction, only the molecules whose level of activity is above a predetermined value E_x are considered functional. The smaller the fraction of functional molecules, the larger is the information content. The size of the arrow is not commensurate with the triangle size.

If the specified degree of function (Ex) is displayed by one trillion different sequences, the functional information content is:

$[I(E_x)] = -\log_2 [10^{12}/10^{390}] = 1255.7$ bits

If a large fraction e.g. 10^{330} sequences (1 sequence in every 10^{60} sequences) show the specified degree of function (Ex), we have:

$[I(E_x)] = -\log_2 [10^{330}/10^{390}] = 199.3$ bits

Should even more, one sequence of every 10^{20} sequences, possess the specified activity, the functional information content of the system would be about 66 bits.

It is important to note that the functional information defined in this manner is meaningful only in the context of a specific function x. Therefore, experimental study of the functional information residing in a set of biological macromolecules is possible only when the researchers decide in advance what the specific function x is, and how they will experimentally measure it. Furthermore, the functional information is always the property of the whole set of molecules, even if that set contains just a single functional molecule, and it is always calculated from a *ratio*.

Studies with RNA

Historically, studies of this kind have been first carried out with RNA, where the specific function was either binding to a ligand or catalysis of a reaction

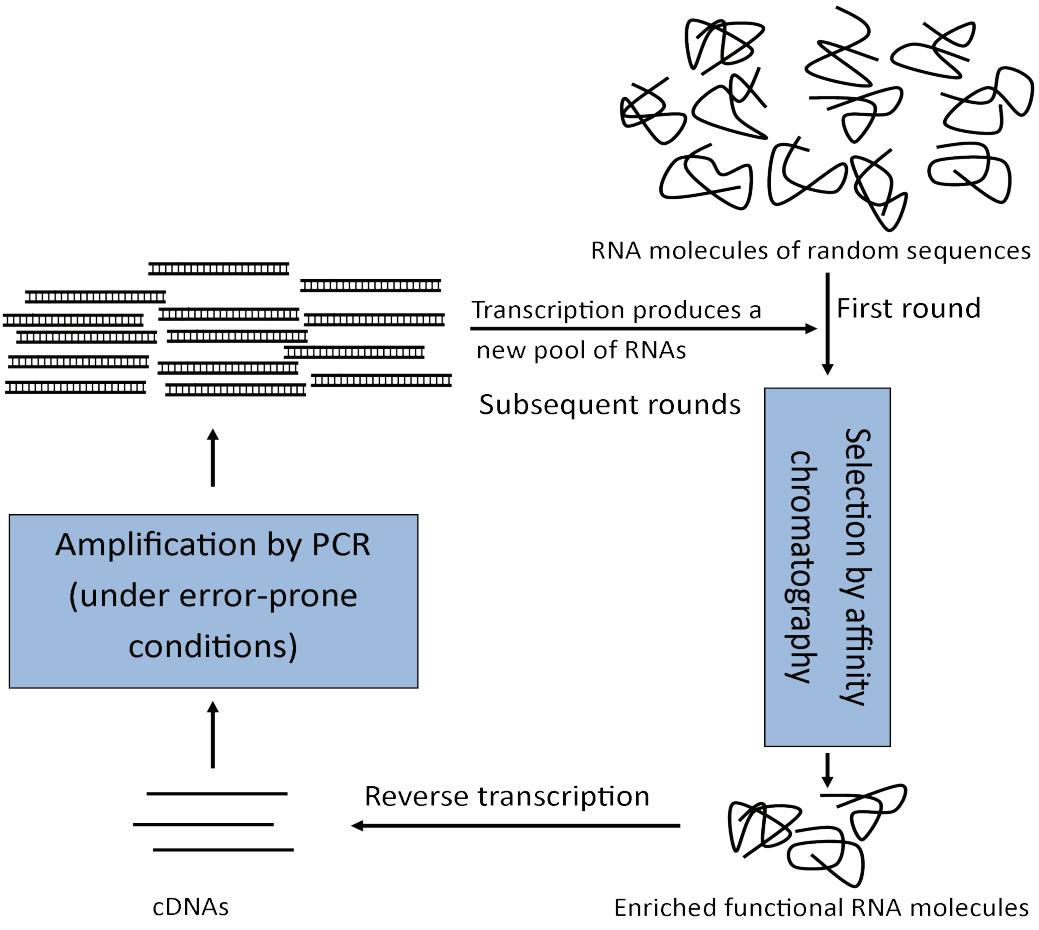

Figure 2
Schematic representation of the method used for the isolation of functional RNA molecules (aptamers). The starting library contains chemically synthesized RNA molecules of similar or identical length but random sequences. They are passed through an affinity chromatography column containing an immobilized ligand. Unbound RNAs are discarded, while those bound to the ligand are eluted and retained. Reverse transcription of these functional RNAs gives cDNAs, which are first amplified by PCR and then transcribed to a new pool of RNAs. Copying errors during PCR introduce new variations into this pool. In the next round at the affinity chromatography step the conditions are made more stringent than in the first round in order to select only those molecules that bind stronger. The stringency is gradually increased in subsequent rounds to select the best functional molecules.

[6-9]. As schematically illustrated in Figure 2, from a starting population of molecules, which was by chemical synthesis intentionally made as heterogeneous as possible in terms of their sequences, selected are only those that possess a defined level of binding or catalysis. Then such RNAs are reverse-transcribed to cDNA. This population of molecules is multiplied by PCR, usually under error-prone conditions to increase variation. The transcription then produces a new set of RNAs enriched in functional molecules. These are then subjected to additional rounds of selection, reverse transcription, amplification and transcription. As the number of rounds increases, the conditions during the selection step are made more and more stringent, so that only the molecules that bind stronger or catalyze better are selected.

In the experiments of this kind, the starting population has usually numbered 10^{13} - 10^{15} molecules, and after a certain number of rounds, usually about 10, researchers have successfully isolated the RNAs capable of binding to various ligands, and catalyzing various reactions, some of which do not occur in nature [10]. The frequency of the functional RNAs capable of binding (called aptamers) was thus estimated to be 1 in about 10^{10} among random sequences, whereas the frequency of the catalytic RNAs (called ribozymes) was about 1 in 10^{13} of random sequences [9].

In a detailed study of the informational content of RNAs capable of binding GTP, it was found that structures of higher complexity were needed in order to bind GTP strongly; a 10-fold tighter binding required about 10 additional bits of information [11]. This was equivalent to specifying the identity of five additional nucleotide positions and corresponded to about a 1000-fold decrease in abundance in a set of random sequences. The best aptamer was 69 nucleotides long, and had the information content of 65 bits [11]. Using the formulae above, one can calculate that the maximal information content was 138 bits, as the RNAs of this length may come in 4^{69} different sequences.

Experiments with proteins

Similar experimental studies with proteins became possible thanks to a major methodological breakthrough which enabled a mRNA molecule to remain covalently linked, after translation, to the protein whose sequence it encoded [12, 13]. One key advantage of this method is that researchers could start with a large population of different DNA molecules, similar in size to that used for selecting functional RNAs. The second major advantage is that the selection of functional proteins automatically results in the selection of their mRNA, allowing for cycles of reverse transcription, PCR amplification, transcription, translation and new selection.

Using this procedure, Keefe and Szostak examined the frequency of functional proteins among polypeptides whose amino acid sequences were almost random [14]. They were not fully random since the authors designed the starting library by a method that increased the number of full open reading frames (ORF) by about 2 orders of magnitude [15]. The function selected for was binding to ATP, and the length of the proteins was 80 amino acids. Four families of ATP-binding proteins were isolated from the starting library of $6x10^{12}$ sequences, indicating that roughly 1 in 10^{11} of the starting sequences possessed ATP-binding activity. According to the authors: "In conclusion, we suggest that functional proteins are sufficiently common in protein sequence space (roughly 1 in 10^{11}) that they may be discovered by entirely stochastic means, such as presumably operated when proteins were first used by living organisms."

For several reasons we disagree with the above conclusion. **First**, a general conclusion is drawn based on a single instance. **Second**, the 2 orders of magnitudes gained in the pre-selection for open reading frames are not counted. When counted, the abundance becomes 1 in 10^{13}. **Third**, proteins that bind ATP represent in current database the largest family of all protein families: the ATP binding cassette transporter family (ABC_tran, PF00005) has 363,409 sequences [16]. Moreover, the distribution of ligands among protein domains and folds follows a power law, so that many ligands bind only to a single protein partner while some ligands bind to many domains and folds: of the 2,186 ligands studied, 1,833 ligands (83.9%) were found to bind to only one fold, 185 (8.5%) were bound to two, while 24 ligands (1.1%) were bound to more than 10 folds [17]. The most common ligand, ATP, binds to proteins of 35 different folds. Furthermore, all nine ligands that bind to more than 15 protein folds are nucleotide ligands [17]. In the BioLiP protein-ligand database which includes over 200,000 ligands, the ligands that bind to a single protein represent by far the largest group [18]. The above described distribution of ligands among protein folds is explained by the intrinsic preference of certain classes of molecules to bind to natural proteins because they are built up of the defined set of 20 amino acids, and this property is exploited at pharmaceutical companies when researchers design novel drugs [17]. Thus, the protein function Keefe and Szostak selected for is not a typical one, but a very frequent one. It is necessary to make a correction for this; given the currently

known distribution [17, 18] we suggest that a ligand binding to ten to thirty protein sequences might be considered as typical. If we add these 4 orders of magnitude (about 300,000/30), the estimate becomes 1 in about 10^{17}.

This estimate is six orders of magnitude lower compared to the estimate of Keefe and Szostak for the abundance of protein functions among random sequences. According to them, the abundance of functional proteins is comparable to the 1 in 10^{11} abundance of aptamers [9], which we consider unrealistic. In contrast to a typical RNA polymer which readily folds into a soluble secondary structure [9, 19], in a typical set of random polypeptides a large fraction does not fold or even precipitates [20-22]. Therefore, the suggested comparable abundance (1 in 10^{11}) for both classes of macromolecules should look suspicious on this basis alone. The fraction of 1 in 10^{11} would indicate that an information content of just 36.5 bits is sufficient for specifying a functional protein of 80 amino acids, where the maximal information content is 345.8 bits. Note that RNAs of equal length (80 nt) would have a maximal information content of 160 bits.

Would we then agree that the abundance of functional proteins among random sequences is 1 in 10^{17}? No, this fraction is still much too high. The protein length of 80 amino acids taken by Keefe and Szostak is much shorter than the average protein length, which is 300 amino acids. Therefore, the size of the sequence space they partially sampled was correspondingly smaller, 10^{104} versus 10^{390}, expectedly making higher the probability of finding a functional protein, if such a function is to be found at all among the proteins that short. Moreover, a protein that is found functional *in vitro* should be tested and proved to function also *in vivo*, as it may be toxic or even lethal *in vivo*. Exactly such an effect was observed when other researchers expressed the ATP-binding protein of Keefe and Szostak in a cell: it created problems and killed the cells [23]. Thus, contrary to the above quoted conclusion, the experimental data show that if this ATP-binding protein were formed stochastically in nature, the living organism that had made it would have left no descendants.

Regulatory function increases complexity

Why would a protein that is functional *in vitro* be lethal *in vivo*? There are many possible reasons, but one of them we view as the most important. The hallmark of biochemical systems is regulation. In a cell, the function of each protein is regulated through interactions with other cell components. In the majority of cases these other cellular components contain proteins, or are proteins themselves. Often a protein that binds a ligand releases that ligand when another protein interacts with it. No such additional function was selected for in the protein of Keefe and Szostak. In recent times, the investigation of protein-protein interactions (PPI) has become an area of intense research. It is out of scope of this paper to provide an overview of this research field, known also as the study of the interactome. We will mention only a few interesting developments.

The same mRNA display method useful for the investigation of the abundance of functional sequences can be adapted to study PPI [24]. The networks formed of protein-protein interactions can be analyzed in terms of 14 motifs that represent functional units of biological processes [25]. The number of possible protein-protein interactions in a cell is so large that compared to it the numbers delineating protein sequence space become negligible. Thus, Tompa and Rose calculated that a protein of 400 amino acids could have on its surface 3,540 distinguishable interfaces capable of interacting with other proteins, and that in a cell with 4,500 different proteins the number of possible interactions is between 10^{7200} and $10^{5.4x10exp7}$ [26]. Therefore, the probability that a novel protein isolated from a set of random sequences will interact in a detrimental manner with other proteins in the cell is unimaginably higher than that it will interact in a beneficial manner.

In the crowded environment of the cell such non-functional interactions constrain the proteome size and gene expression [27, 28]. The estimated number of actual PPI in the human interactome varies between 130,000 and 600,000 [29], so that a protein interacting with 4-5 partners is considered moderately connected [30]. At the high end there are proteins like p53 that interact with over 100 other proteins [31]. Evidently, the amino acid sequence of a protein must specify also the information for regulation of its activity through interactions with other proteins. This means that the representation of functional information as shown in Figure 1 is incomplete, and that a true picture must take into account also protein-protein interactions, as shown in Figure 3.

Regulated activity as bits of information

Hence to talk about a function of a protein today means talking about a regulated activity. To the scientists of earlier generations the picture appeared much simpler, as extensively reviewed by Braun and Gingras [32]. How many additional bits of information

are needed for specification of a typical protein-protein interaction? Tompa and Rose assume that on the average 22 amino acids on protein surface take part in such interactions [26], which would correspond to a

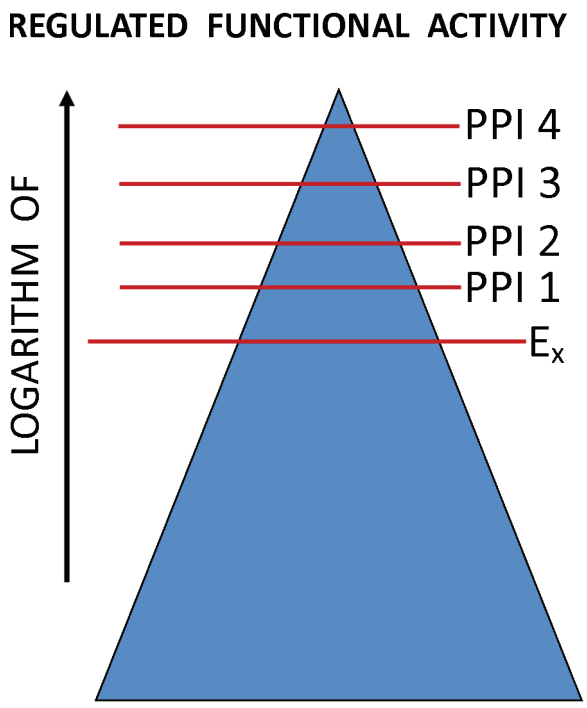

Figure 3
A more realistic schematic representation of functional information content that is present among proteins of random sequences. There are many protein sequences that satisfy the requirement for a functional activity above a predetermined value Ex, but a vast majority of them will not function properly in a given cell. In the cell, functional activity of proteins is regulated largely through interactions with other proteins, so we encounter there regulated functional activity. After taking account of the necessary protein-protein interactions (PPI), the fraction of properly functioning sequences sinks dramatically.

maximal information content (no mutations allowed at any of the 22 positions) of 20^{22}, or 95.1 bits. If so, a protein of 80 amino acids would allow for three such non-overlapping PPI (285.2 bits) and still have about 60.4 bits left to specify its functional activity. If 95 bits for one PPI seem too high, let us see how low we can go. Recently, Anand *et al.* found that the majority of binding sites had 15-20 residues in them [33]. Taking the lower value, 20^{15} would correspond to 64.8 bits, or to the probability of 1 in 3.3 x10^{19} for finding such a site in a random sequence. Perhaps even a single less stringent PPI could make the ATP-binding protein of Keefe and Szostak non-lethal, one could argue, or perhaps several much less stringent PPI would do. In protein interaction networks, the number of interaction partners depends on the distance a particular protein is from the central hubs, where the distribution of the hubs is non-random [34]. In yeast, an ATP binding cassette transporter has six protein binding partners [35], whereas the mean number of partners is 4.7 [36]. The shortest binding sites – and very few of such ones are known – contain just 3 amino acids [37]. If we assume that 4 binding sites each of only 3 amino acids, or, equivalently, one site of 12 amino acids, would make the ATP-binding protein of Keefe and Szostak non-lethal, that would correspond to 51.9 bits, or the probability of 1 in 4.2 x 10^{15}.

So, what is finally the fraction of functional proteins among random sequences? We doubt there is a single meaningful figure applicable to proteins of all lengths and functions. For the reasons outlined above, we insist that *in vivo* data are needed for such estimates in order to take account of PPI. Following the calculations above, if we take account of the about 1 in 10^{15} figure together with the above derived 1 in 10^{17}, we arrive at 10^{-32} as the fraction of functional proteins. The 10^{-15} value for specifying PPI of a novel protein derived from a random sequence appears reasonable in view of the experimental finding that after mutating a few residues in originally fully regulated functional enzyme, triose phosphate isomerase, only one out of 10^{10} *in vitro* active mutants functioned properly in cell context [38].

The 10^{-32} fraction conservatively derived here is significantly higher than some earlier estimates. Thus, in 1979 Yockey, based on reported cytochrome c sequences, estimated that this fraction is 10^{-65} [39]. Reidhaar-Olson and Sauer estimated in 1990 that the fraction is 10^{-63} [40]. Later Axe concluded from his studies with penicillin degrading beta-lactamases that the probability of finding a functional enzyme among random sequences is about 10^{-77} – 10^{-53} [41]. In a study of four large protein families, Durston and Chiu estimated that functional sequences occupy an extremely small fraction of sequence space, in all cases lower than 10^{-100} [42]. On the other hand, the estimate of Taylor *et al.* is that a library of 10^{24} members should contain an AroQ mutase [43]. In view of these different figures, and given the paucity of experimental data at present, we are convinced that one is on the safe side by saying that, until proven otherwise, the fraction of functional proteins among random sequences is *lower than* 1 in 10^{20}.

The meaning of estimates

But of what importance are these estimates? In the article "Chance and Necessity in the Selection of Nucleic Acid Catalysts" Lorsch and Szostak explain it [9]:

"In Tom Stoppard's famous play, the ill-fated heroes toss a coin 101 times. The first 100 times they do so the coin lands heads up. The chance of this happening is

approximately 1 in 10^{30}, a sequence of events so rare that one might argue that it could only happen in such a delightful fiction. Similarly rare events, however, may underlie the origins of biological catalysis. What is the probability that an RNA, DNA, or protein molecule of a given random sequence will display a particular catalytic activity? The answer to this question determines whether a collection of such sequences, such as might result from prebiotic chemistry on the early earth, is extremely likely or unlikely to contain catalytically active molecules, and hence whether the origin of life itself is a virtually inevitable consequence of chemical laws or merely a bizarre fluke."

Along this line of thinking, there are thus profound philosophical ramifications connected with the abundance of functional proteins among random sequences. If that fraction is below 1 in 10^{30}, to believe that functional proteins arose by chance would be a "delightful fiction". Those scientists who prefer the first answer to Socrates' question will be philosophically inclined to maintain this fraction as high as possible for all proteins. On the other hand, those scientists who prefer the second answer will tend to consider that the existence of a single functional protein of, say not higher than 10^{-50} abundance among proteins of random sequences, proves the inadequacy of chance and necessity. Neither the first nor the second group of scientists has become convinced by the arguments of the other side till now.

Can discovery of singletons solve the impasse?

At first sight it seems that the impasse will continue for many years until all scientists come to agree on a single figure. And it might also seem that our conservative setting of an *upper limit* at 10^{-20} serves only to prolong this impasse. However, that is not the case thanks to experimental data from another field, genome sequencing. Researchers have sequenced the genomes of several thousand species thus far, and as noted by Kozulic [44] the single most surprising result is the finding that in each genome there are hundreds, or even thousands, of novel unique proteins called singletons, so unique that they show no homology above the level displayed by proteins of random sequences. If the probability of finding one functional protein among random sequences is at most 10^{-20}, then the probability that a cell acquires two such proteins is at most 10^{-40}, three proteins 10^{-60}, etc. It is irrelevant in which generation of a *lineage* the acquisitions take place: the formation of every new singleton is associated with the overcoming of the low probability of its formation (10^{-20}). Here we consider only this aspect and for simplicity disregard all the aspects related to population genetics. Such simple calculation is justified because the acquisitions of singletons by one lineage are *connected* and *independent* events [45, 46]. They are connected because they must happen in one lineage, whereas they are independent because the acquisition of one singleton does not depend on the acquisition of another singleton. This calculation brings us quickly from the area of plausibility into the area of "delightful fiction". It is easy to see that 10 orders of magnitude up or down in the probability of finding functional proteins among random sequences play only a minor role in view of such a high number of singletons that have been discovered in every sequenced genome. Concerning random sequences, there is a deep insight, with which we agree, expressed by Keefe and Szostak [14]:

"*Unlike other libraries that have been used in protein selections, this random region is not part of a larger structure that would otherwise tend to constrain or bias the conformation of the resulting proteins. This library randomly samples the whole of sequence space, rather than the vicinity of a known protein.*"

Random protein sequences do sample the whole of sequence space. And by experiments researchers can only sparingly sample the protein sequence space owing to its large size: thus, for the synthesis of a library containing all the proteins that are just 100 amino acids long one would need to use all the atoms from 10^{23} universes of the size of our own universe [47]. In view of this, how shall we interpret the discovery of hundreds of unique proteins, of the sequences so unrelated that they are undistinguishable from random, in all sequenced genomes? Is it possible to avoid the conclusion that they come from far away "regions" of the protein sequence space? The discovery of how many such unique proteins – one thousand, one million or one billion – is needed to convince a reasonable person that the whole of protein sequence space was actually searched?

Selection of enzymes from a partially randomized non-catalytic scaffold

Using their powerful mRNA display technology, Seelig and Szostak started with a 460 amino acid long sequence of a stable domain of human protein retinoid-X-receptor that naturally binds to DNA with participation of two zinc finger loops [48]. They randomized these two small loop sections of this protein

(altogether 21 amino acids), and produced a library of 10^{12} randomly created sequences. The authors then selected those sequences that were capable of joining two RNA oligonucleotides, of which one had a 5'-triphosphate, and another a free 3'-hydroxyl group. After 17 rounds of PCR amplification, transcription, translation, reverse transcription and selection, they obtained ligases providing catalytic rate enhancement of more than two-million fold compared to the just metal-catalyzed reaction.

The reported result looks impressive until we compare the performance of the ligases created by Seelig and Szostak with natural ligases. A kinetic study of a ligase was recently published [49]. T4 DNA ligase working on nicked duplex DNA has a kcat approaching 1 s^{-1} and a Km of about 3 nM. The ligase of Seelig and Szostak has a kcat of about 1 h^{-1}, which is 3600-fold lower compared to the natural enzyme. While the authors did not report the Km value, they stated that 10 micromolar substrate is subsaturating, meaning that the Km is at least that high. This Km value is again about 3000-fold worse than that of the T4 ligase. The ratio of kcat/Km, which is generally used to characterize the efficiency of an enzyme, of their ligase is therefore about 10-million-fold worse than that of T4 ligase.

The poor efficiency of the created ligase is especially noteworthy in view of the fact that one of the substrates contained an energy-rich 5'-triphosphate group. In contrast, natural enzymes first bind and react with ATP before joining two DNA or RNA ends, of which 5'-end contains a phosphate and 3'-end a hydroxyl group. Owing to these differences in reaction mechanism, there is little room for doubt that their enzyme would not be functional in a living cell, had it been by chance formed *in vivo*. Moreover, when expressed in a cell, five of the seven ligases aggregated or precipitated, even though only relatively small stretches, covering only 21 of 460 amino acids, were modified. This finding is in line with many reports of researchers engaged in protein engineering, and it strengthens the notion about the importance of PPI.

In the literature one often encounters a statement that natural selection can little by little, over many generations, improve an initially poor function, so that just the first step leading to emergence of a new function is really critical. As far as we know, this view has not been subject to critical experimental examination when the function relates to or relies on proteins, and most cellular functions do so. The only paper – to our knowledge – is that of Nasvall *et al*. in which the authors claim that formation of a new gene is associated with multiple duplications of an existing gene [50]. However, their existing gene product already functioned in the cell context. Furthermore, under their experimental conditions the multiple gene copies could have increased the protein concentration by about one order of magnitude. The compensation for poor specific activity by an increase in protein expression level may work only up to a certain protein concentration. Beyond that concentration, non-functional interactions of many proteins with the protein whose concentration is extremely high, say one million fold to compensate for its one million fold lower activity, become so prevalent that the cell ceases to function properly, as discussed by Zhang *et al*. [27].

In terms of functional information, if just one of the 20^{21} (~2 $x10^{27}$) randomized sequences possessed the desired ligase activity, the maximal information content would be 90.8 bits. In the first round Seelig and Szostak started with about 10^{12} molecules, a vast majority of which was of unique sequence, so we can assume that the information content was about 40 bits. In subsequent rounds, however, the majority of molecules were exact copies of those molecules selected in earlier rounds. It would be complicated to estimate the number of new sequences in each round in order to calculate the increase in information content. For our purpose it is sufficient to know that starting with 40 bits it could not have reached 90 bits. It is tempting to try another route in order to extract the same information. The reported experimental data, and their Figure 2, show that in the first 8 rounds the fraction of selected molecules was about 0.01%. So, a single molecule was selected and 9,999 discarded out of each group of 10,000 molecules. This corresponds to an increase of the information content of about 13.3 bits per round, giving cumulatively 8x13.3=106.4 bits. Of course, this figure is an overestimate because the calculation assumes that all molecules in each round are novel sequences. But the calculation is useful because it gives us a feeling of the possible maximal gain in information at each round of selection.

It is worth noting that in the closing section of the paper the authors did not claim that their results gave credence to the view that enzymes can be formed from enzymatically inactive proteins starting from random mutations that are subsequently fixed in a population by genetic drift or natural selection. Such an evolutionary perspective was provided in a comment of Seelig and Szostak's paper published in the same issue of the journal [51]. Now we will show why that perspective is fundamentally erroneous.

Do laboratory experiments mimic evolution?

Commenting on Seelig and Szostak's work, Robertson and Scott say that "the authors create an artificial evolution process" and that they bypass "by simulating evolution" our lack of understanding on how the amino acid sequence dictates the structure and catalytic properties of a protein [51]. However, they have given us no clues as to how the experimental data of Seelig and Szostak could fit into a model that would simulate an evolutionary process. This lack of connection between biochemical data of this sort and evolutionary modeling is the rule rather than an exception in contemporary publishing practice of the major scientific journals.

Ever since Roland Fisher introduced population thinking into evolutionary modeling [52], such thinking has gained acceptance among evolutionary biologists, so that today few of them would disagree with Michael J. Lynch that "Nothing in evolution makes sense except in light of population genetics" [53]. Once a mutation appears in a population, it can spread in that population mainly by two processes: natural selection or/and genetic drift. Changes in the abundance of neutral mutations are generally governed by random genetic drift, while the abundance of advantageous mutations is increased by natural selection. The artificial selection employed by Seelig and Szostak [48], as well as by Keefe and Szostak [14], has some resemblance to natural selection, whereas it has no resemblance to genetic drift. Therefore, let us consider their experimental data in view of natural selection.

Comparison with real populations

As noted before, the starting library from which the ATP-binding proteins and RNA ligases were selected contained about 10^{12} different DNA molecules. To think in evolutionary terms, we need to assume that each one of these DNA sequences represents a part of the genome of a single living organism in a population. The population would then evidently consist of 10^{12} individuals, each having a segment of different sequence in its DNA (plus other usual variations). Would such a population correspond to any real population that could have lived during the history of the earth? No, it would not. The differences in DNA sequences among individuals of our hypothetical population would be much larger – and more focused – compared to the differences in DNA sequences in the genomes of individuals of real populations. Specifically, in the library of Keefe and Szostak the whole 240 bp long DNA sequence (encoding 80 amino acids) was randomized. In contrast, such a randomized stretch of genomic (or plasmid) DNA cannot be produced by any process that exists in nature, neither in one living individual, nor in two living individuals that could act as parents. In the library of Seelig and Szostak two targeted short segments, of 63 bp cumulatively (21 amino acids), were randomized; again, no natural process can do this. If from these hypothetical starting populations the genomes of any two individuals were sequenced and compared, the differences in the 240 bp segment would be counted at hundreds of positions, whereas in the segments of 63 bp they would be counted at tens of nucleotide positions.

In contrast, two unrelated human individuals typically differ at only about 1 position in a stretch of 1000 bp. Therefore, the level of genetic variation across the selected short segments in the genomes of individuals from these hypothetical populations would be many times higher than is possible in any real life population. Thanks to this high level of DNA variation in the starting population of 10^{12} molecules, the probability of finding a functional sequence was much higher there than it could have ever been in a population of 10^{12} living individuals. Here we have the ***first*** major difference between the laboratory work with molecules and the natural processes that work on individuals in populations.

Selection and fixation

Let us proceed without any attempt to compensate for the high variation in the laboratory experiments, for example by increasing the population size. After the first selection step, Seelig and Szostak retained 0.01% of the starting population of 10^{12}: in the population genetics terms this would mean that 0.1 billion individuals survived natural selection whereas 999.9 billion were eliminated. Subsequent PCR amplification would correspond to an expansion of the selected population. According to the detailed description of the experimental protocol [54], the amount of DNA after the PCR amplification was about 10-fold lower compared to the starting 10^{12} molecules. In population genetic terms, this would mean that the population has expanded from 0.1 billion to about 100 billion. Speaking in the same terms, the fixation of desired alleles in that population, and in all subsequent ones, would be close to 100%. If we consider only the first 8 rounds, and apply straightforward addition, the number of eliminated individuals would come to about 1,800 billion.

The straightforward summation applied above, however, is not the proper way of calculating. The fixation of an allele under natural selection in a real life population depends on its selection coefficient, that is, on the strength of the benefit conferred by the new allele. The stronger the benefit, the higher the probability that such an allele will spread in the population and get fixed. For example, in population genetics modeling, the selection coefficient of 0.01 is considered as a strong one. However, according to John H. Gillespie, the probability of fixation of a new allele with such a selection coefficient is only about 2 % [55]:

"Equation 3.23 tells us, for example, that a new mutation with a 1 percent advantage when heterozygous, hs = 0.01, has only a 2 percent chance of ultimately fixing in the population. A 1 percent advantage represents rather strong selection. In a very large population, say N = 10^6, 1 percent selection will overwhelm drift once the allele is at all common. Yet, 98 percent of such strongly selected mutations are lost."

Here is the ***second*** major difference between the laboratory experiments and the real life situation. In the laboratory, essentially 100% fixation was ensured instantly. What would be a reasonable value for selection coefficients of Seelig and Szostak's peculiar ligases during each one of the 17 rounds of selection, which ended with an enzyme of the efficiency about 10 million-fold lower compared to natural ligases, especially in the first rounds when the enzyme activity was very low? One can only guess. Let us be generous and take 0.005 for all rounds; then the fixation probability would be 1%. How shall we correlate this factor with the laboratory protocol? We can imagine the existence of 100 populations so that the fixation becomes essentially certain in one of them. And if we take account, in order to simplify the calculation, of only 8 of the 17 rounds each with 100 populations, then the number of individuals that would have been eliminated by natural selection increases 100-fold, to about 180,000 billion. Lower, more realistic selection coefficients would of course result in even higher numbers of eliminated individuals.

One way of putting the above number in a perspective is this: according to the Population Reference Bureau the estimated number of individuals belonging to the species *Homo sapiens* who have ever been born is 108 billion [56]. According to the above calculation, this figure is 1670-fold lower than the number of individuals that natural selection would need to have at its disposal for elimination in order to create a new enzyme of poor efficiency. The species *Homo sapiens* is biologically a successful one when measured by its population size: many other mammalian species have never reached, and will never reach, such a large population. In such species natural selection would be even more limited in creating functional novelty that relies on new proteins: simply put, the needed raw material – individuals in a population – were not available to it.

The above simplified calculations did not include one important consideration: During DNA amplification by PCR, DNA mutation rate is 4-6 orders of magnitude higher compared to a typical mutation rate in organisms of a growing real life population. This is the ***third*** important difference between the *in vitro* and *in vivo* conditions. Such increased variation represents one more factor that has improved the odds of finding a functional protein in the laboratory as opposed to a natural population.

The ***fourth*** important difference between natural populations and laboratory experiments lies in the increasing stringency of the selection conditions in the laboratory. The laboratory selection corresponds to gradual increase of pressure from the environment (climate change, competing species, etc.), always in the same direction over the whole time period during which our hypothetical populations expanded, then contracted, then expanded again, and so on 17 times. How likely is such a steadiness over geological time scales? One can only guess, but this uncertainty was obviously removed in the laboratory protocol.

Scientists have used more resources than available for evolution

Let us summarize: the experimental protocols of Szostak and co-workers differ in four important aspects from natural processes that lead to the appearance and spreading of mutations in natural populations. Two of them, the randomized sequences and overly high mutation rates during PCR, directly provided more DNA variation among molecules than could have ever been present among individuals in a natural population. The two other differences, the increased selection pressure and immediate fixation in the laboratory, resulted in immense saving of the material undergoing selection.

To illustrate this last point, assuming a selection coefficient of 0.005, in each round Seelig and Szostak should have performed 100 PCR experiments, altogether 1,700 PCR experiments, each with 200 samples to get about 1 ml of DNA solution according to their protocol [54]. Then they should have discarded the product of 99 of them, and continued with the protocol using the remaining one.

Obviously, all four different factors combined give a tremendous advantage to the laboratory protocol. After correlating the laboratory protocol with population genetics, our simplified calculations, always intentionally tilted in favor of evolution, show that in the laboratory the researchers have worked with such a huge number of individual molecules that the corresponding number exceeds for many species the total number of individuals that have ever lived on the earth. Accordingly, natural selection could have never had at its disposal such a huge number of individuals. This leads us to the conclusion that scientists have already experimentally searched wider possibilities than have ever been within the reach of evolutionary processes.

As noted above, in terms of functional information the information content of Seelig and Szostak's initial DNA library was about 40 bits, whereas the maximal final information content was about 90 bits. So, the selection process could have created at most 50 bits of new information. To achieve this meager result, natural selection would have eliminated over 180,000,000,000,000 individuals. The 50 bits of information correspond to about 11 letters in English alphabet (26^{11}). According to Hazen *et al*., an analogy exists between the functional information of macromolecules and language [1]. Thus, to write something equivalent to DESIGNWORKS or NOEVOLUTION a species would need to pay natural selection with the lives of over 180,000 billion of its members.

Discussion

In the preceding sections we have provided detailed comments on the experimental results of Szostak's group and explained in a step-by-step manner why our interpretation of these results is opposite to theirs and to those of Robertson and Scott. Even though this should be evident from the preceding sections of this paper, here we would like to reiterate that our criticism does not relate to their experimental protocols or their published results. We hold in high esteem the originality of their ideas and methodological approaches, and we express our deep appreciation for the amount of experimental work that was necessary to get the results of such a quality and volume.

Now we will address some of the possible arguments against our conclusions. It could be argued, as we have done, that a general conclusion cannot be drawn based on just one or two instances. The parallel, however, is valid only at first sight. In the case of ATP-binding proteins, we have made corrections for the facts that the function selected for is very frequent among known proteins, and that Keefe and Szostak have paid no attention to selection for PPI that should ensure regulation of the activity of this protein in the cell. With these factors included the calculation becomes more realistic and the chance of finding a functional protein among random sequences sinks dramatically.

In the case of RNA ligases, in addition to the four already mentioned advantages in favor of the laboratory protocol as opposed to natural populations, there is a ***fifth*** one: the starting protein already had the substrate-binding activity, so that only the catalytic activity was searched for. And the ***sixth*** advantage is this: since the starting retinoid-X-receptor protein already functioned in the cell, the surface of this protein was already capable of functional PPI. Accordingly, if in the future researchers decide to look for a new enzyme activity among random sequences, their chances of finding one will be dramatically lower. For these reasons we are confident that our conclusions stand.

It might be argued that no correction for instantaneous fixation is needed, or that our calculations are wrong. For example, instead of 100 populations of the original size (10^{12}), why not imagine a division of the starting population into 100 sub-populations? In such a scenario, however, the chance of finding the functional activity in one sub-population would be 100-fold lower (remember the size of the protein sequence space!). And the corresponding laboratory protocol would also then have to handle 100 PCR experiments for each round. One could further argue that no correction is needed because of the essential certainty of fixation in a situation where many beneficial alleles are present in a population [55, p. 95]. But the existence of this situation in the population of molecules is precisely one important point that differentiates it from natural populations. To properly account for this difference we would need to include a factor much larger than 100. The situation where 100,000,000 mutations all appear simultaneously and are directed to a single function can exist only in the laboratory and in fiction.

It is possible to criticize our conclusions in view of the fact that they are based on simplified calculations, rather than on population genetics modeling. However, there is simply no published model that would in population genetics terms adequately reflect the experimental protocols of Szostak and coworkers. On the other hand, a paper of extraordinary importance, in our opinion, was published recently by Chatterjee *et al.* [57]. The authors address a topic highly relevant to ours: the time scale that is needed for evolutionary

innovations. Their key finding is that for arriving at functional sequences largely different from the starting one the processes take exponential time in sequence length. For a typical sequence of 1000 nucleotides, the search does not succeed in geological time even if multiple populations are searching at the same time, when the search starts from a flat point in the fitness landscape: exactly the situation which exists when starting from a random sequence. The search is unsuccessful even when the target area is very broad:

"The estimated number of bacterial cells on earth is about 10^{30}. To give a specific example let us assume that there are 10^{24} independent searches, each with population size N~10^{6}. The probability that at least one of those independent searches succeeds within 10^{14} generations for sequence length L=1000 and broad peak of c =1/2 is less than 10^{-26}."

The search is unsuccessful also when the targets are many and broad, regardless of which population size is modeled [57, and supplement]. This study thus supports our conclusion that there is a strict limit to what the commonly understood evolutionary processes can achieve in terms of new functional proteins of the sequences so unrelated that they appear random.

Probability limit

Of course we are not the first ones to argue that there is a limit to what evolutionary processes can achieve. For example, Michael Behe in *The Edge of Evolution* and in subsequent work has provided strong evidence that such an edge exists [58]. John Sanford in *The Genetic Entropy* and elsewhere has explained not only why natural selection is poor at creating novelty in genomes, but also why it is incapable of preventing genome deterioration [59]. Douglas Axe has given strong evidence why just a single new protein fold remains beyond the reach of evolutionary processes [60], and why the novelty these processes can introduce into proteins in terms of amino acid positions can be counted by the fingers in one hand [61]. Are we then of the opinion that future studies like those of Szostak and co-workers are superfluous? By no means! But we are convinced that their results will only strengthen the conclusion we have expressed here.

In earlier sections of this paper the word probability has occurred so many times that we suspect there will be readers, especially among philosophers and metaphysicians (are the two not dwelling in each one of us?), who will exclaim: enough of probability, give us certainty! Here is a part of the story a metaphysician told Bertrand Russell (from his *Metaphysician's Nightmare*):

"There is a special department of Hell for students of probability. In this department there are many typewriters and many monkeys. Every time that a monkey walks on a typewriter, it types by chance one of Shakespeare's sonnets. There is another place of torment for physicists. In this there are kettles and fires, but when the kettles are put on the fires, the water in them freezes. There are also stuffy rooms. But experience has taught the physicists never to open a window because, when they do, all the air rushes out and leaves the room a vacuum."

In our world, the phenomena of low probability happen seldom or never, while the phenomena of high probability happen frequently or always. This makes our world predictable. Today little discussion goes on about the phenomena that happen always or frequently because, as noted by Bertrand Russell, the truly interesting ones are those that are improbable but not impossible. Can one determine where the transition from improbable to impossible takes place? Others have said a lot on this topic [62, 63], so here we will not treat it at all, but we only wish to express our agreement with Lorsch and Szostak that an origin of a functional biological macromolecule whose probability of happening on the earth is below 10^{-30} ought to be considered impossible.

Conservation of information

In recent times a lot of discussion has been ongoing regarding the *Conservation of Information* (CoI) theorem, which states [64]:

"… CoI applies to search, showing that searches must employ existing information to successfully locate targets, and that locating targets through search never outputs more information than was inputted into the search initially. Searches, in finding targets, output information. At the same time, to find targets, searches need to input information. CoI shows that the output cannot exceed the input. "

The experimental work of Szostak and coworkers provides, in our opinion, a nice *practical* demonstration of the validity of the Conservation of Information theorem. Thus, the search for functional RNA ligases among partially randomized amino acid sequences delivered at most 50 bits of new functional informa-

tion, whereas the detailed specification of the method of search – in the article by Burckhard Seelig: "mRNA display for the selection and evolution of enzymes from in vitro-translated protein libraries" – required 368,144 bytes x 8 = 2,945,152 bits of PDF file [54]. Just the title contains 395 bits (84 letters without spaces). The information content of the file without figures (53,465 characters, no spaces) would be 231,069 bits. It is true that in that protocol not all of information relates directly to the search method, but it is also true that the proper use of the protocol relies on additional information that the researchers possess related to the use of instruments, as well as related to other uses of information acquired during their specialized education.

It is thus evident that the input of functional information has exceeded the output by many orders of magnitude. One can argue that we are comparing apples with oranges, as the functional information in proteins is different from the functional information in language. We disagree because, to continue the analogy, we are not comparing apples with oranges but the mass of the apples with the mass of the oranges. This is legitimate because 1 kg is 1kg in both cases; so is 1 bit. Should the critics insist that the functional information must be of the same kind – whatever that may mean – our reply is: then the search for a novel functional protein can be done only by an old functional protein; and that is absurd.

Some philosophical considerations

We shall conclude this paper with some thoughts that will help our readers place the preceding sections in a broader context. Our goal is also to help our philosophical opponents see us as we see ourselves, and see themselves as we see them. We are aware that many biologists will consider us as mere disturbers of the theoretical order of their field, and so they will most likely choose to ignore our writings as the strategy to deal with them. In doing so, however, they overlook that ignoring the experimental findings about which we write will not make these findings go away or lose their importance.

Two opposing views

Others will recognize in us disturbers of their view of the living world, and ascribe it to our lack of understanding of the modern evolutionary theory. In so doing they implicitly assume that one must agree with everything one understands. However, that assumption is false. Some readers may regard as unfair our use of scientific data for the criticism of a philosophical position of others. Should not cherished philosophical positions, personal beliefs and convictions be spared from such criticism? Let us see how Friedrich Nietzsche (1844-1900) addressed this question (in: *The Gay Science*):

"In science, convictions have no right to citizenship, as one says with good reason: only when they decide to step down to the modesty of a hypothesis, a tentative experimental standpoint, a regulative fiction, may they be granted admission and even a certain value in the realm of knowledge — though always with the restriction that they remain under police supervision, under the police of mistrust. But doesn't this mean, on closer consideration, that a conviction is granted admission to science only when it ceases to be a conviction? Wouldn't the cultivation of the scientific spirit begin when one permitted oneself no more convictions? That is probably the case; only we need still ask: in order that this cultivation begin, must there not be some prior conviction — and indeed one so authoritative and unconditional that it sacrifices all other convictions to itself? We see that science, too, rests on a faith; there is simply no 'presuppositionless' science. The question whether truth is necessary must get an answer in advance, the answer 'yes', and moreover this answer must be so firm that it takes the form of the statement, the belief, the conviction: 'Nothing is more necessary than truth; and in relation to it, everything else has only secondary value.' This unconditional will to truth — what is it? Is it the will not to let oneself be deceived? Is it the will not to deceive? …. So, the faith in science, which after all undeniably exists, cannot owe its origin to such a calculus of utility; rather it must have originated in spite of the fact that the disutility and dangerousness of 'the will to truth' or 'truth at any price' is proved to it constantly. 'At any price': we understand this well enough once we have offered and slaughtered one faith after another on this altar!"

The philosophical convictions that prevail in biology today have gained their dominant position by, in Nietzsche's terms, "slaughtering" other philosophical convictions, as every educated person knows, in the name of scientific truth. For this reason those same prevailing convictions of today cannot ask for clemency; and furthermore, there is no one who could grant them such a clemency. Science moves forward in complete disregard of everyone's philosophical convictions, cherished or not.

Science as judge

We are aware that some philosophers have argued, and will continue to argue that the relationship between science and philosophy as Nietzsche, we and many others understand it is wrong because, they will contend, it is not science that judges philosophy, but philosophy that judges individual sciences. We deny the validity of this objection. But with an important qualification: science cannot judge which philosophical position is true, but it can judge which philosophical position is false. Every such judgment is of course philosophical, and not scientific. In our opinion, the acceptance of this view was the necessary condition for the birth of modern science in Europe. That view is possibly best expressed in a single sentence of Leonardo da Vinci (1452-1519) (in: *Notes*, 1157):

"The man who blames the supreme certainty of mathematics feeds on confusion, and can never silence the contradictions of sophistical sciences which lead to an eternal quackery."

Later this same view was expressed, further developed and reduced to practice by Galileo Galilei (1564-1642), and many others who followed in their footsteps. The current paper is not the right place to review or discuss the history of science even in a cursory manner; here we shall instead take advantage of several key words from the above pregnant sentence of Leonardo da Vinci.

The above sentence was penned at the time when discussions on some philosophical questions had continued, according to written records available to Leonardo, for over 2,000 years. No wonder then that Leonardo says such discussions could continue forever without resolution, and because of that they are nothing else but quackery. Many scientists of our time, us included, would agree with Leonardo, perhaps with minor reservations.

If a philosophical question can be associated or analyzed in the terms that can be treated mathematically, then of the two contradictory answers to the philosophical question one can be silenced thanks to the supreme certainty of mathematics. Again, we believe that most scientists of today, including us, would agree with Leonardo. And isn't a person refusing to accept the certainty of a mathematical proof feeding on confusion, and thus responsible for the continued existence of the confusion?

Now we have come face to face with the crucial issue: How is it possible that the scientists who are in agreement regarding results of a mathematical treatment of a set of experimental data relevant to a philosophical question, can nevertheless totally disagree about which one of the two contrary philosophical answers is true? To resolve this issue we need to look closer at Leonardo's "*contradictions of sophistical sciences*".

Fictitious arguments

The term sophistical has several different, but related, meanings described in detail in Plato's *Sophist* (we like the translation by Benjamin Jowett). Thus:

"The philosopher he cannot be, for upon our view he is ignorant; but since he is an imitator of the wise he will have a name which is formed by an adaptation of the word sophos. What shall we name him? I am pretty sure that I cannot be mistaken in terming him the true and very Sophist." And further: *"And may there not be supposed to be an imitative art of reasoning? Is it not possible to enchant the hearts of young men by words poured through their ears, when they are still at a distance from the truth of facts, by exhibiting to them fictitious arguments, and making them think that they are true, and that the speaker is the wisest of men in all things?"*

Do we wish to suggest that the plausibility of one of the two contradictory answers is maintained by fictitious arguments? Yes, we do; but in place of a lengthy logical exposition, let us address this question with help of practical examples and real-life situations familiar to scientists.

When a chemist completes the synthesis of a novel compound, what makes him sure that he has actually obtained that specific compound and not any one of the millions of other possible compounds? The chemist would reply: I know which reactants I took for the reaction and they limit the number of possible compounds that can be formed; further, I subjected the reaction product to several powerful purification procedures and obtained one major component; and finally, the purified compound was subjected to elemental analysis, polarimetry, ^{1}H-NMR, ^{13}C-NMR, IR and HPLC-MS, and all the data are consistent with my structural formula for the compound. You are still not convinced? Well, show me then your alternative formula that will not contradict any of my analytical results. Here the discussion would end; not in one instance or two, but in about 50,000,000 instances, once for each of the about 50,000,000 different compounds which chemists have synthesized to date.

The supreme certainty of the chemist rests on the principle of non-contradiction. The principle was first formulated by Aristotle (384 – 322 B.C.E.), who built

on criticism of the sophists by Socrates and Plato. This first principle of logic states that, in the words of Aristotle, *"it is impossible that contradictories should be at the same time true of the same thing"* where *"a contradiction is an opposition which of its own nature excludes a middle"*. Another well-known formulation is: *"Nothing can be, and not be, at the same time."* Thus, for example, when a substance is identified as sulfuric acid then it cannot not be sulfuric acid at the same time. This may sound trivial, but it is not, as we shall see immediately.

Hegelian logic

The principles of Aristotelian logic were further developed and remained essentially unchallenged for over 2,000 years till the early nineteenth century, when Hegel (1770-1831) in his "*The Science of Logic*" attacked them head-on. This quote from Wallace's translation (p. 223) serves as an illustration:

"Instead of speaking by the maxim of Excluded Middle (which is the maxim of abstract understanding) we should rather say: Everything is opposite. Neither in heaven nor in earth, neither in the world of mind nor of nature, is there anywhere such an abstract 'Either-or' as the understanding maintains. Whatever exists is concrete, with difference and opposition in itself. The finitude of things will then lie in the want of correspondence between their immediate being, and what they essentially are. Thus, in inorganic nature, the acid is implicitly at the same time the base: in other words, its only being consists in its relation to its other. Hence also the acid is not something that persists quietly in the contrast: it is always in effort to realise what it potentially is. Contradiction is the very moving principle, of the world: and it is ridiculous to say that contradiction is unthinkable."

We believe our comments would be of no help in making clearer the clash with Aristotelian logic. But it is necessary to emphasize this: For Aristotelian logic a contradiction is what a stop codon is for the ribosome, what the end of the road is for the car driver, and what land is for the sea captain; ignoring the contradiction leads inevitably to disaster. In contrast, for Hegelian logic a contradiction is the very moving principle of the world, it is everywhere the fuel that powers development; and so there is no reason to worry about when faced with a contradiction. As Benjamin Jowett, the translator of Sophist and himself a disillusioned Hegelian, said:

"To the Hegelian all things are plain and clear, while he who is outside the charmed circle is in the mire of ignorance and 'logical impurity': he who is within is omniscient, or at least has all the elements of knowledge under his hand."

The contemporary biochemistry represents a battlefield on which these two incompatible logical systems clash: the biochemists coming from the chemistry side are largely Aristotelians, while those coming from the biology side are often Hegelians. No possibility exists for convincing a consistent Hegelian that he is in error, simply because every proof rests on the principle of non-contradiction, which our Hegelian not only denies, but builds on that denial his picture of the world. On the other hand, it is hardly surprising that Aristotelians find all kinds of logical errors in the arguments that Hegelians use to justify their convictions. Now we shall address some exemplars of these errors.

***Contradictio in adjecto* in modern biochemistry**

In relation to the topic of this paper, the most frequently encountered logical error is known as *contradictio in adjecto* (CIA), in which the meaning of the adjective stands in opposition to the meaning of its noun. The often used classic example is *wooden iron*. One can easily see why it is a logical error. Just try to answer this question: Will *wooden iron* sink or float? Any of the two answers can be justified, and challenged, in so many different ways that the only possible outcome can be Leonardo's eternal quackery. When confronted with a *wooden iron*, only one question is allowed: Does it exist? The answer No prevents all the quackery.

The term *directed evolution* is a similar *contradictio in adjecto*. One can see this by asking the question: Will *directed evolution* move toward a certain goal? One can answer: Yes, because otherwise it would not be directed. But one can also answer: No, because the hallmark of Darwinian evolution is the absence of any preferred direction [65-71].

The adjective directed generally implies intention and therefore intelligence; but the noun evolution in its meaning excludes intention and intelligence. Because two contrary answers cannot both be true, but here both are allowed to be true, the term *directed evolution* does not denote anything truly existing in nature; it is therefore a meaningless phrase for natural scientists. But why would anyone coin a meaningless phrase? Let us analyze an example of its role:

"*Protein engineering is a method of generating altered proteins with desirable properties and can be pursued by two general strategies. The first approach is a rational design that uses detailed knowledge of the structure and function of a protein to make useful changes to the amino acid sequence. The second approach harnesses the power of natural selection to modify the protein's properties and is known as directed evolution.* "[54]

Here the role of "directed evolution" is to make it distinct from "rational design". This second phrase represents also a *contradictio in adjecto*; the placing of adjective "rational" in front of "design" implies that design can be irrational too (see below). But that is contradictory to all generally accepted definitions of design, some of which are described in the Introduction. In the text quoted above, one CIA is opposed to another CIA in an attempt to hide the evident, which is this: both scientific approaches represent design, pure and simple. But when so, obviously, there is no place for natural selection.

The above sophistical reasoning allows its followers to believe that the type of experiments reviewed in this paper mimic or simulate the processes that take place in nature, when in fact such experiments *probe the limits* of these processes. This fact, however, makes no sense at all in the philosophical system of all those who believe that everything living today is the result of those processes. For them, those processes can have no limit that scientists could probe.

There are numerous other *wooden irons* of the same kind intended to serve the same purpose: to blur the distinction between design and evolution, or even to equate the two. Here we will mention a few exemplars, like "evolutionary strategy", "evolutionary design", "coordinated evolution", "laboratory evolution" and "*in vitro* evolution", but particularly noteworthy is "irrational design". Some authors even choose these CIAs for the title or key words [72-74]. A quick search of the literature with these CIAs will reveal thousands of papers. We see no point in dealing further with them here. But we cannot refrain from dealing with an extreme case: "intrinsically disordered proteins". The two adjectives, *intrinsically* and *disordered* serve to deny what every natural protein molecule by its very nature is: a polymer of amino acids *ordered* in a perfectly defined way, the order being determined by the protein primary amino acid sequence, and equally determined by its corresponding mRNA and DNA sequences. This fundamental truth of biochemistry is well expressed by Francis Crick [75]:

"*To produce this miracle of molecular construction all the cell need do is to string together the amino acids (which make up the polypeptide chain)* ***in the correct order***" [boldface is ours; in the original these words were in italics].

Therefore, a better case for identifying *contradictio in adjecto* with logical error can hardly be found. Let us now see the practical consequences:

"Alternatively spliced protein segments tend to be intrinsically disordered and contain linear interaction motifs and/or posttranslational modification sites. An emerging concept is that differential inclusion of such disordered segments can mediate new protein interactions, and hence change the context in which the biochemical or molecular functions are carried out by the protein. Since genes with disordered regions are enriched in regulatory and signaling functions, the resulting protein isoforms could alter their function in different tissues and organisms by rewiring interaction networks through the recruitment of distinct interaction partners via the alternatively spliced disordered segments." [76]

Accordingly, something *disordered* contains "interaction motifs" and "modification sites", "is enriched in regulatory and signaling functions" and can recruit "distinct interaction partners". In short, disorder brings function. We raise our voice against such a corruption of biochemistry, the science to which we have devoted a large part of our lives. And for the corruption we put the blame squarely on the intrusion of Hegelian logic. We are not the first ones to address philosophical implications of "intrinsically disordered proteins" [77]. This term was not chosen by accident among many options – of which some like "partially folded" reflect the reality – but with the intention to forcefully associate the *concept* of disorder with proteins, as explicitly stated by Uversky [78].

The concepts that scientists use to describe a part of reality they investigate reflect their philosophical views. Recently, David Snoke pointed out how in the field of systems biology the scientists employ many teleological concepts, like design, information processing and optimization. In spite of the fact that these concepts belong to the intelligent design (ID) view of biology, many contemporary scientists who use these concepts apparently still believe that they continue to work within the Darwinian research program [79]. Their belief is maintained by the confusion regarding the true meaning of terms, a problem we discussed above related to biochemistry.

Contradictions – to be ignored or resolved?

Our readers with philosophical background will recognize that in the above discussion on the meaning of terms we have taken the position of logical positivists, in particular Schlick [80]. Specifically, we share his views that in answering certain questions there is no difference between the method of philosophy and special sciences, and that the meaning of a claim is clear to us only when we are able to state accurately the conditions that must be satisfied if the claim is to be true. If the conditions are not satisfied, then the claim is false. In other words, the criterion for the truth or falsity of the proposition then consists in the absence or presence of certain data under specific circumstances.

Why do many biologists simply ignore the experimental data that contradict their established theory? Such behavior is to be expected from all those who follow contradictions-tolerant Hegelian logic. In the recently published dispute between two streams of evolutionary biologists [81], one cannot find a single sentence about the fact that genome sequencing has uncovered enormous number of novel genes and proteins. Our writing about these experimental results will likely be labeled as hostile to science by evolutionary biologists. But we wonder if they really do not see that such a conclusion is derived from a false equation: science = contemporary evolutionary theory.

How can one explain the current situation? Is Nietzsche perhaps right again (from: *A Genealogy of Morals*, Hausemann's translation)?

"Oh, how much is to-day hidden by science! Oh, how much it is expected to hide! The capacity of our best scholars, their inconsiderate industry, their head reeking, fuming, day and night, their handicraft-mastery: — how often all this finds its ultimate sense in the fact that they wish to hide something from themselves! Science as a means of self-narcosis — ye know of that?"

Many of our colleague scientists will likely feel a strong urge to run away from all these philosophical issues by summarily declaring, with Stephen Hawking: "*Philosophy is dead!*" We answer with Etienne Gilson's quote: "*Philosophy always buries its undertakers*". To find out why, it is necessary to read philosophy. We recommend reading the original texts of the major philosophers – most of them are freely available on the Internet – rather than the writings of their followers, adversaries, or commentators. After some time you will start feeling that Ph.D. means more than before when associated with your name.

We shall conclude this paper by commenting the last two sentences of the *On the Origin of Species*:

"*Thus, from the war of nature, from famine and death, the most exalted object which we are capable of conceiving, namely, the production of the higher animals, directly follows. There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.*"

The quintessential question, however, is not whether there is *grandeur* in this view of life, but whether there is *truth* in this view of life. Our resolute answer is No; there is no truth in that view of life, based on several independent lines of published experimental results. The data reviewed here are sufficient for the conclusion that the war of nature, famine and death, which would eliminate hundreds of trillions of individuals, could at best produce just a single protein of poor functional activity outside of the cell and of no functional activity inside the cell.

References

1. Hazen RM, Griffin PL, Carothers JM, Szostak JW (2007) Functional information and the emergence of biocomplexity. Proc Natl Acad Sci 104:8574-8581. doi:10.1073/pnas.0701744104

2. Szostak JW (2012) Attempts to define life do not help to understand the origin of life. J Biomol Struct Dyn 29(4): 599-600. doi:10.1080/073911012010524998

3. Szostak JW (2003) Functional information: Molecular messages. Nature 423(6941): 689-689. doi:10.1038/423689a

4. http://biochemical-pathways.com/#/map/1

5. Brocchieri L, Karlin S (2005) Protein length in eukaryotic and prokaryotic proteomes. Nucleic Acids Res 33: 3390-3400. doi:10.1093/nar/gki615

6. Huizenga DE, Szostak JW (1995) A DNA aptamer that binds adenosine and ATP. Biochemistry 34(2): 656-665. doi:10.1021/bi00002a033

7. Lorsch JR, Szostak JW (1994) In vitro selection of RNA aptamers specific for cyanocobalamin. Biochemistry 33(4): 973-982. doi:10.1021/bi00170a016

8. Lorsch JR, Szostak JW (1994) *In vitro* evolution of new ribozymes with polynucleotide kinase activity. Nature 371(6492): 31-36. doi:10.1038/371031a0

9. Lorsch JR, Szostak JW (1996) Chance and necessity in the selection of nucleic acid catalysts. Acc Chem Res 29(2): 103-110. doi: 10.1021/ar9501378

10. Wilson C, Szostak JW (1995) *In vitro* evolution of a self-alkylating ribozyme. Nature 374(6525): 777-782. doi:10.1038/374777a0

11. Carothers JM, Oestreich SC, Davis JH, Szostak JW (2004) Informational complexity and functional activity of RNA structures. J Amer Chem Soc 126(16): 5130-5137. doi:10.1021/ja031504a

12. Roberts RW, Szostak JW (1997) RNA-peptide fusions for the *in vitro* selection of peptides and proteins. Proc Natl Acad Sci 94(23): 12297-12302

13. Nemoto N, Miyamoto-Sato E, Husimi Y, Yanagawa H (1997) *In vitro* virus: bonding of mRNA bearing puromycin at the 3'-terminal end to the C-terminal end of its encoded protein on the ribosome in vitro. FEBS letters 414(2): 405-408. doi:10.1016/S0014-5793(97)01026-0

14. Keefe AD, Szostak JW (2001) Functional proteins from a random-sequence library. Nature 410(6829): 715-718. doi:10.1038/35070613

15. Cho G, Keefe AD, Liu R, Wilson DS, Szostak JW (2000) Constructing high complexity synthetic libraries of long ORFs using *in vitro* selection. J Mol Biol 297(2): 309-319. doi: 10.1006/jmbi.2000.3571

16. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, *et al*. (2013) Pfam: the protein families database. Nucleic acids research, 1-9. doi: 10.1093/nar/gkt1223

17. Ji HF, Kong DX, Shen L, Chen LL, Ma BG, Zhang HY (2007) Distribution patterns of small-molecule ligands in the protein universe and implications for origin of life and drug discovery. Genome Biol, 8: R176. doi:10.1186/gb-2007-8-8-r176

18. http://zhanglab.ccmb.med.umich.edu/BioLiP/

19. Moore PB (1999) The RNA folding problem. Cold Spring Harbor Monograph Series 37: 381-402

20. Beasley JR, Hecht MH (1997) Protein design: the choice of *de novo* sequences. J Biol Chem 272(4): 2031-2034. doi: 10.1074/jbc.272.4.2031

21. Blanco FJ, Angrand I, Serrano L (1999) Exploring the conformational properties of the sequence space between two proteins with different folds: an experimental study. J Mol Biol 285(2): 741-753. doi:10.1006/jmbi.1998.2333

22. Tanaka J, Takashima H, Yanagawa H (2010) Comparative characterization of random-sequence proteins consisting of 5, 12, and 20 kinds of amino acids. Prot Sci 19(4): 786-795. doi:10.1002/pro.358

23. Stomel JM, Wilson JW, León MA, Stafford P, Chaput JC (2009) A man-made ATP-binding protein evolved independent of nature causes abnormal growth in bacterial cells. PloS one 4(10): e7385. doi:10.1371/journal.pone.0007385

24. Fujimori S, Hirai N, Ohashi H, Masuoka K, Nishikimi A, *et al*. (2012) Next-generation sequencing coupled with a cell-free display technology for high-throughput production of reliable interactome data. Sci Rep. 2:691. doi:10.1038/srep00691

25. Tran NH, Choi KP, Zhang L (2013) Counting motifs in the human interactome. Nature Comm 4: 2241. doi:10.1038/ncomms3241

26. Tompa P, Rose GD (2011) The Levinthal paradox of the interactome. Prot Sci 20(12): 2074-2079. doi:10.1002/pro.747

27. Zhang J, Maslov S, Shakhnovich EI (2008) Constraints imposed by non-functional protein–protein interactions on gene expression and proteome size. Mol Syst Biol 4:210. doi:10.1038/msb.2008.48

28. Levy ED, De S, Teichmann SA (2012) Cellular crowding imposes global constraints on the chemistry and evolution of proteomes. Proc Natl Acad Sci 109(50): 20461-20466. doi:10.1073/pnas.1209312109

29. Kuzmanov U, Emili A (2013) Protein-protein interaction networks: probing disease mechanisms using model systems. Genome med 5(4): 1-12. doi:10.1186/gm441

30. Bonetta L (2010) Protein-protein interactions: Interactome under construction. Nature 468(7325): 851-854. doi:10.1038/468851a

31. Huang Y, Jeong JS, Okamura J, Sook-Kim M, Zhu H, Guerrero-Preston R (2012) Global tumor protein p53/p63 interactome. Cell Cycle 11(12): 2367-2379. doi:10.4161/cc.20863

32. Braun P, Gingras A C (2012) History of protein–protein interactions: From egg-white to complex networks. Proteomics 12(10): 1478-1498. doi:10.1002/pmic.201100563

33. Anand P, Nagarajan D, Mukherjee S, Chandra N (2014) PLIC: protein–ligand interaction clusters. Database, bau029. doi: 10.1093/database/bau029

34. Fadhal E, Gamieldien J, Mwambene EC (2014) Protein interaction networks as metric spaces: a novel perspective on distribution of hubs. BMC systems biology 8(1): 6. doi:10.1186/1752-0509-8-6

35. Paumi CM, Menendez J, Arnoldo A, Engels K, Iyer KR, *et al*. (2007) Mapping protein-protein interactions for the yeast ABC transporter Ycf1p by integrated split-ubiquitin membrane yeast two-hybrid analysis. Molecular Cell, 26(1): 15-25. doi:10.1016/j.molcel.2007.03.011

36. Babu M, Vlasblom J, Pu S, Guo X, Graham C, *et al*. (2012) Interaction landscape of membrane-protein complexes in Saccharomyces cerevisiae. Nature 489: 585–589. doi:10.1038/nature11354

37. Neduva V, Linding R, Su-Angrand I, Stark A, De Masi F, *et al*. (2005) Systematic discovery of new recognition peptides mediating protein interaction networks. PLoS biology, 3(12): e405. doi: 10.1371/journal.pbio.0030405

38. Silverman JA, Balakrishnan R, Harbury PB (2001) Reverse engineering the (β/α) 8 barrel fold. Proc Natl Acad Sci 98(6): 3092-3097. doi:10.1073/pnas.041613598

39. Yockey HP (1977) A calculation of the probability of spontaneous biogenesis by information theory. J Theor Biol 67(3): 377-398. doi:10.1016/0022-5193(77)90044-3

40. Reidhaar-Olson JF, Sauer RT (1990) Functionally acceptable substitutions in two α-helical regions of λ repressor. Proteins 7(4): 306-316. doi:10.1002/prot.340070403

41. Axe DD (2004) Estimating the prevalence of protein sequences adopting functional enzyme folds. J Mol Biol 341:1295-1315. doi:10.1016/jmb.2004.06.058

42. Durston KK, Chiu DK (2012) Functional Sequence Complexity in Biopolymers. In: Abel D, ed, The First Gene: The Birth of Programming, Messaging and Formal Control, LongView Press – Academic, pp.117-133

43. Taylor SV, Walter KU, Kast P, Hilvert D (2001). Searching sequence space for protein catalysts. Proc Natl Acad Sci 98(19): 10596-10601. doi:10.1073/pnas.191159298

44. Kozulic B (2011) Proteins and genes, singletons and species. http://vixra.org/pdf/1105.0025v1.pdf

45. Boole G (1854) An Investigation of the Laws of Thought. Walton and Maberly (London), pp. 243-252 (Chapter XVI)

46. Venn J (1962) The logic of chance. Fourth Edition, Chelsea Publishing Company (New York), pp. 1-22.

47. Wei Y, Hecht MH (2004) Enzyme-like proteins from an unselected library of designed amino acid sequences. Prot Eng Des Sel 17(1): 67-75. doi:10.1093/protein/gzh007

48. Seelig B, Szostak JW (2007) Selection and evolution of enzymes from a partially randomized non-catalytic scaffold. Nature 448(7155): 828-831. doi:10.1038/nature06032

49. Lohman GJS, Chen L, Evans TC (2011) Kinetic Characterization of Single Strand Break Ligation in Duplex DNA by T4 DNA Ligase. J Biol Chem 286: 44187–44196. doi: 10.1074/jbc.M111.284992

50. Näsvall J, Sun L, Roth JR, Andersson DI (2012) Real-time evolution of new genes by innovation, amplification, and divergence. Science 338(6105): 384-387. doi:10.1126/science.1226521

51. Robertson MP, Scott WG (2007) Biochemistry: designer enzymes. Nature 448(7155): 757-758. doi:10.1038/448757a

52. Fisher RA (1930) The genetical theory of natural selection. Oxford: Clarendon Press, 1930

53. Lynch M (2007) The frailty of adaptive hypotheses for the origins of organismal complexity. Proc Natl Acad Sci 104 (Suppl 1): 8597-8604. doi:10.1073/pnas.0702207104

54. Seelig B (2011) mRNA display for the selection and evolution of enzymes from *in vitro*-translated protein libraries. Nature protocols 6(4): 540-552. doi:10.1038/nprot.2011.312

55. Gillespie JH (2010) Population genetics: a concise guide. JHU Press (Baltimore) p 94

56. http://www.prb.org/Publications/Articles/2002/HowManyPeopleHaveEverLivedonEarth.aspx

57. Chatterjee K, Pavlogiannis A, Adlam B, Nowak MA (2014) The Time Scale of Evolutionary Innovation. PLoS computational biology 10(9): e1003818. doi: 10.1371/journal.pcbi.1003818

58. Behe MJ (2010) Experimental evolution, loss-of-function mutations, and "the first rule of adaptive evolution". Q Rev Biol 85(4): 419-445. doi:10.1086/656902

59. Nelson CW, Sanford J (2013) Computational evolution experiments reveal a net loss of genetic information despite selection. In: Marks II RJ, Behe MJ, Dembski WA, Gordon BL, Sanford JC, eds. Biological Information—New Perspectives. World Scientific (Singapore) pp 338-368

60. Axe DD (2010) The case against a Darwinian origin of protein folds. BIO-Complexity 2010(1):1-12. doi:10.5048/BIO-C.2010.1

61. Axe DD (2010) The limits of complex adaptation: an analysis based on a simple model of structured bacterial populations. BIO-Complexity 4: 1-10. doi:10.5048/BIO-C.2010.4.c

62. Dembski WA (2004) The logical underpinnings of intelligent design. In: Dembski WA, Ruse M, eds. Debating design: From Darwin to DNA, Cambridge University Press, 311-330

63. Abel DL (2009) The universal plausibility metric (UPM) & principle (UPP). Theor Biol Med Model 8:27. doi:10.1186/1742-4682-6-27

64. Dembski WA (2014) Being as Communion, Ashgate Pub Co (Burlington) pp 151-152

65. Darwin C (1890) The variation of animals and plants under domestication. D. Appleton & Co (New York) pp 428-429

66. Huxley T (1880) The lay sermons. D. Appleton & Co (New York) pp. 301, 303

67. Haeckel E (1901) The riddle of the universe at the close of the nineteenth century. Harper & Brothers (New York, London) pp 270

68. Dawkins R (1996) The blind watchmaker. WW Norton & Co (New York) pp 21

69. Crick F (2004) Of molecules and men. Prometheus Books (New York) pp 7

70. Dobzhansky T (1975) Darwinian or 'Oriented' Evolution? Evolution 29: 376-378

71. Morgan TH (1910) Chance or Purpose in the Origin and Evolution of Adaptation. Science 31(789): 201-210

72. Tobin MB, Gustafsson C, Huisman GW (2000) Directed evolution: the 'rational' basis for 'irrational' design. Curr Opin Struct Biol 10(4): 421-427. doi: 10.1016/S0959-440X(00)00109-3

73. Schuster P (2013) Designing living matter. Can we do better than evolution? Complexity 18(6): 21-33. doi:10.1002/cplx.21461

74. Arnold FH (1998) Design by directed evolution. Acc Chem Res 31(3): 125-131. doi:10.1021/ar960017f

75. Crick F (1981) Life itself, Its Origin and Nature. Simon and Schuster (New York) pp 51

76. Buljan M, Chalancon G, Dunker AK, Batema A, Balaji S, *et al*. (2013). Alternative splicing of intrinsically disordered regions and rewiring of protein interactions. Curr Opin Struct Biol (3): 443-450. doi:10.1016/j.sbi.2013.03.006

77. Goodwin W (2011) Structure, function, and protein taxonomy. Biol Philos 26(4): 533-545. doi:10.1007/s10539-011-9252-8

78. Uversky VN (2013) A decade and a half of protein intrinsic disorder: Biology still waits for physics. Protein Sci 22(6): 693-724. doi:10.1002/pro.2261

79. Snoke K (2014) Systems Biology as a Research Program for Intelligent Design. BIO-Complexity 2014 (3):1–11. doi:10.5048/BIO-C.2014.3

80. Schlick M (1979) Positivism and realism. In Vienna Circle Collection, ed. Mulder HL, ed. Kluwer pp 259-284

81. Laland K, Uller T, Feldman M, Sterelny K, Müller GB, Moczek A, *et al*. (2014) Does evolutionary theory need a rethink? Nature 514(7521): 161-164. doi:10.1038/514161a

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