
# Advertising science for high impact publication

# Advertising Science for High Impact Publication

Stefan Franzen[1,*] and Keith T. Gagnon[2]

[1]*Department of Chemistry, North Carolina State University, Raleigh, NC 27695, USA*

[2]*Departments of Pharmacology and Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA*

**Abstract:** An examination of the scientific literature shows a tendency to promote results rather than simply report them. The difference is often subtle and yet important for the perceived scientific value. This is particularly true for publication in journals with a high impact factor, where it is important to succinctly define novelty and immediate significance. The marketing of scientific results is often justified as a necessity to obtain funding in a competitive climate. The ethical issue surrounding the use of scientific language arises when the promotion of discovery blurs or masks accurate reporting. In this study, we examine the use of scientific language that capitalizes on popularized ideas, discoveries or technologies to enhance the perceived impact of published observations. A case study provides an example of how careful use of scientific language might help maximize publication impact, yet result in misleading or inaccurate reporting of data that adversely affects the publication as well as the research community.

**Keywords:** Buzzwords, scientific language, scientific marketing, impact factor, case study, SELEX, ribozyme, Diels-Alder.

## THE USE OF POPULAR LANGUAGE AND FAIR SCIENTIFIC MARKETING

Most can agree that if a novel chemical transformation or biological process is observed, then a clear description and careful documentation should be sufficient for publication. However, if the authors wish to impress upon the reader that the result is not merely interesting but a process or finding that has special attributes, then the authors might employ the use of fashionable scientific buzzwords to enhance the perceived value of their observation. Exaggerated use of buzzwords in science often correlates with the emergence of a popularized discovery, technology or idea in the scientific community. Examples of words with this quality might include "evolutionary", "genome-wide", or the many words with the prefix "nano". Such words can be continually popular, like "evolutionary", or newly minted to cater to funding opportunities, such as "nano" (Fig. **1**). Others correspond to a clear advance in technology, as is the case for "genome-wide". Genome-wide was simply not in use before the advent of microarray hybridization technologies, which were introduced on a practical scale in the late 1990s [1-3]. Within the last few years deep sequencing technology, capable of reading millions of nucleic acid fragments and mapping sequences back to the entire genome, has bolstered the use and popularity of this word [4-6]. The use of words such as "nanoparticle" in place of the older "colloid" coincided with the advent of the National Nanotechnology Initiative, which is a major funding directive in several federal agencies [7]. The occurrence of a certain scientific buzzword in the literature can grow at an unprecedented rate as scientists adapt their research focus to stay current with hot topics (Fig. **1**). The shift can be driven by the desire to remain relevant in a research field and to obtain support in an often unforgiving funding climate. Thus, a sharp increase in the use of a popular scientific term in the literature could be expected to reflect a combination of more research and more reviews on related topics as well as some variable level of inflated use.

The use of popular or well-recognized words to describe or define results in publication does not appear to carry any inherent ethical issues. Indeed, the ability to responsibly incorporate trendy scientific buzzwords into a manuscript might be considered a desirable writing skill [8-10]. This is simply scientific marketing in its most basic form, which aims to present science in terms that are more readily appreciated and understood by a broader audience. We use the term marketing to mean promotion of the scientific idea and its importance without a value judgment. Marketing does not in itself compromise ethics. In fact, marketing may be viewed as an important asset in the economy of scientific research, where publications are the products or services produced by laboratories while journals, funding agencies, public media and pharmaceutical and industrial companies are the distributors and consumers [11, 12]. The use of popular scientific language and terminology is an integral part of communication and dissemination of research progress in our modern society. However, as in any commercial enterprise, the integrity of the scientific process depends on truth in advertising. How does one determine when marketing is no longer honest?

The point at which responsible use of scientific language and buzzwords devolves into unethical hype to improve impact or exposure is often blurry, subjective and difficult to define. Unqualified use of popular scientific jargon or buzzwords is usually transparent to journal editors and reviewers. It is detected early enough for correction since the words

*Address correspondence to this author at the Department of Chemistry, North Carolina State University, Raleigh, NC 27695, USA;
Tel: (919) 515-8915; Fax: (919) 515-8909;
E-mail: Stefan_Franzen@ncsu.edu

**1874-7612/10** 

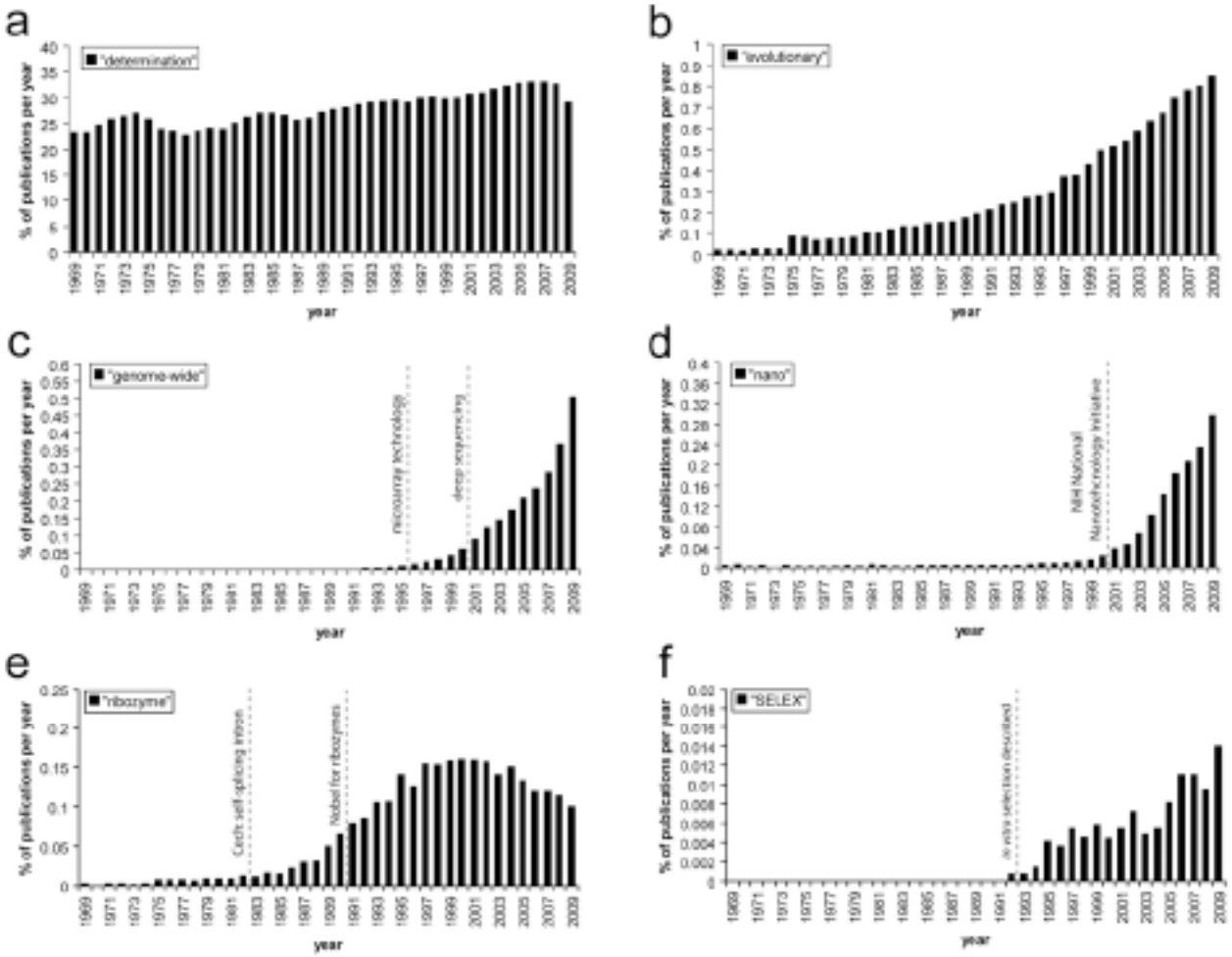



**Fig. (1).** The occurrence of certain popular buzzwords in the scientific literature. Publications retrieved from a PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) search with the designated keyword. Publications are plotted as % of total publications for that year versus the publication year. The commonly occurring scientific keyword "determination" serves as a negative control for this analysis. The word "determination" has no particular application to a field and is not expected to increase with time as a percentage of total citations since. Indeed, this expectation is borne out in panel a, which shows fairly constant usage in about 30% of all articles from 1969 to the present.

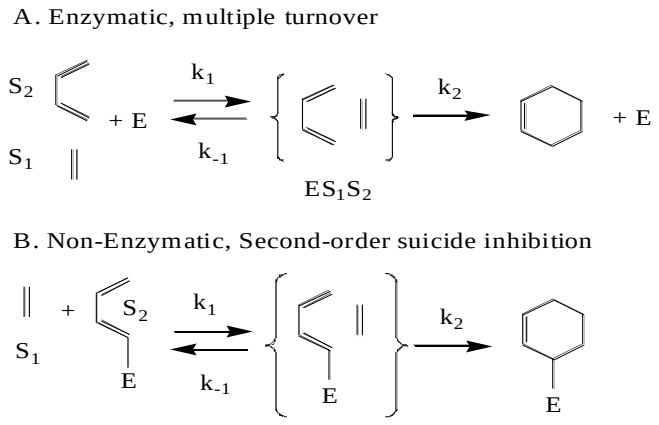
carry with them the expectation of certain experiments or results that warrant their use. Occasionally, however, careful word choice can help propel a set of observations into a scientific category where it might not have otherwise been recognized. If misuse does succeed, it can unfortunately become propagated throughout the literature and research community, further compounding the problem. Moreover, the subtle, improper use of terms runs the risk of leading to false statements if it continues unchecked. Recent examples of gross fraudulence in the stem cell research field illustrate how the pressure to generate influential discoveries can drive researchers to become unscrupulous [13-15]. The topic of ethics in scientific language is rarely discussed yet represents an underdeveloped area in the ethics of science [9, 10, 16, 17]. The training that young scientists receive in writing papers is not standard [18] yet they are nonetheless expected to uphold a uniform, largely unspoken code of ethics in writing and publishing results.

Identifying what may denote irresponsible or inappropriate use of scientific language is both subjective and circumstantial and is best illustrated by a case study. In this approach, we use historical context, our research expertise and experience in writing and publishing science to analyze an article that has remained reputable yet drawn questions concerning acceptable use of scientific terminology and data analysis. Our goal here is not to pass judgment on whether the authors of the article have misused scientific marketing or potentially breeched an ethical code in reporting and publishing their work. Rather, we call attention to the issue by weighing their use of language against the actual data presented. This examination is inherently technical in nature. We recognize that many readers may be unfamiliar with the scientific issues involved, and we have made every effort to translate the science and ethical concerns into common language.

## A CASE STUDY IN THE USE OF SCIENTIFIC LANGUAGE TO MAXIMIZE IMPACT

The article chosen for a case study describes an artificial method for facilitating carbon-carbon bond formation in a reaction known as the Diels-Alder reaction (Scheme **1**) [19]. The article is now quite dated and does not appear to be part of any active research based on a recent assessment of the scientific literature. The creation of new carbon-carbon

bonds is an important process in chemical synthesis, which has important implications for creation of new pharmaceuticals and diagnostic molecules, among other applications. It would be desirable to find new ways to create bonds using catalysts, which are known as enzymes in biology. Catalysts and enzymes accelerate chemical processes, which is an essential feature of all living organisms, but also useful for industrial chemical synthesis. Enzymes that act to accelerate chemical processes in the cell are almost always given a name ending in "ase". For example, a "foldase" would mean an enzyme (catalyst) that accelerates folding, as in protein folding, which is essential for formation of protein structures. Prior to 1982, all known enzymes in biology were proteins. The "central dogma of biology" held that information was stored in deoxynucleic acid (DNA), transcribed to ribonucleic acid (RNA), which was just a messenger, and then the RNA translated into proteins, which were the active components of the cell that carried out the tasks necessary for life. RNA was discovered to have enzymatic activity, to act as a catalyst, first in 1982 [20]. The word ribozyme was coined shortly thereafter to distinguish catalysis by RNAs (ribozymes) from proteins (enzymes). There is no known natural enzyme or ribozyme that can carry out the chemical transformation shown in Scheme **1**, which is known as the Diels-Alder reaction. Thus, any report of an artificial ribozyme with such a capability would be both novel and important.




**Scheme 1.** Illustration of two kinds of transformations that involve the Diels-Alder reaction between a diene, $S_2$, and dienophile, $S_1$. **A**) In the enzymatic transformation the enzyme E is unchanged during the course of the reaction. **B**) In the non-enzymatic transformation the bimolecular reaction between $S_1$ and $S_2$ causes the enzyme to become inactivated.

We now discuss how implying the discovery of a "Diels-Alder ribozyme," or Diels-Alderase (DAase), helped enhance perceived impact but was not qualified on the basis of the data presented. We also suggest that the misuse of language served to undermine both the quality of the data and the progress of the research community. We endeavor to present both sides of the argument, wherever possible, in both a historical and scientific context. This evaluation is not meant to determine the intent of the authors. However, any effort to establish a standard by which to measure the acceptable use of language or buzzwords in reporting science requires an assessment of the appropriateness of claims, as they relate to key words aimed at maximizing impact versus actual experimental results. With this background, we discuss the outcomes of misusing scientific language on the quality of research and the impact it has on the scientific community.

In 1997 the article "RNA-catalysed carbon-carbon bond formation" appeared in *Nature*, arguably the most revered journal in science [19]. The article described an RNA sequence that was selected by a method known as systematic evolution of ligands by exponential enrichment (SELEX) to accelerate the formation of a carbon-carbon bond in a Diels-Alder reaction. The use of 'RNA-catalysed' in the title, as well as 'DAase' throughout, implied the discovery of a novel ribonucleic acid (RNA) enzyme, or ribozyme. At the time of publication, the popularity of SELEX and ribozymes in the scientific literature were both on the rise (Fig. **1**). SELEX had been described only a few years earlier and appeared to hold promise for evolution of high-affinity binding molecules [21-24]. After the Nobel Prize in Chemistry was awarded to Tom Cech and Sid Altman in 1989 for the paradigm-changing discovery of ribozymes, characterization and discovery of ribozymes as well as their general interest skyrocketed [25-27]. Thus, within a few years two new technologies had been discovered based on RNA. One was an evolutionary method to discover new binding sequences and the other was that RNAs could be catalytic, or enzymatic. In addition to these high impact discoveries, a novel enzymatic process to generate carbon-carbon bonds by the well-known Diels-Alder reaction (Scheme **1**) holds special interest due to its potential for widespread use in synthetic chemistry [28-30]. Adding further to the excitement was the claim that an artificial chemical modification of pyridine on the uridine, one of the four nucleotide building blocks of RNA, was essential for the Diels-Alderase activity. The combination of these features appeared to be more than fitting for publication in *Nature* at the time, which routinely publishes articles of exceptional novelty and high impact.

## CECH'S DILEMMA AND THE PROOF OF RNA CATALYSIS

The primary issue in language usage arises from the word 'catalysed' in the article title. A catalyst is defined as "a substance, usually used in small amounts relative to the reactants, that modifies and increases the rate of a reaction without being consumed in the process" (www.thefreedictionary.com). In other words, a catalyst facilitates the reaction but is essentially unchanged in the process so that it can accelerate additional reactions. By the standard definition, the RNA described in the 1997 *Nature* paper is not a catalyst since it is chemically altered (now covalently attached to the dienophile substrate) and cannot be recovered or reused to accelerate subsequent reactions (Fig. **2**). The counter-argument to this point is that this method is designed only as a first step and that the principle of carbon-carbon bond formation, once demonstrated, can be used to design a catalyst by separating the diene substrate from the RNA. At the time of publication, there was a historical precedent for believing that this would indeed be the case, as well as a prevailing optimism in the field that allowed this shortcoming to be brushed aside.

When Cech first reported 'catalytic' properties of the *Tetrahymena thermophila* self-splicing intron RNA in 1982

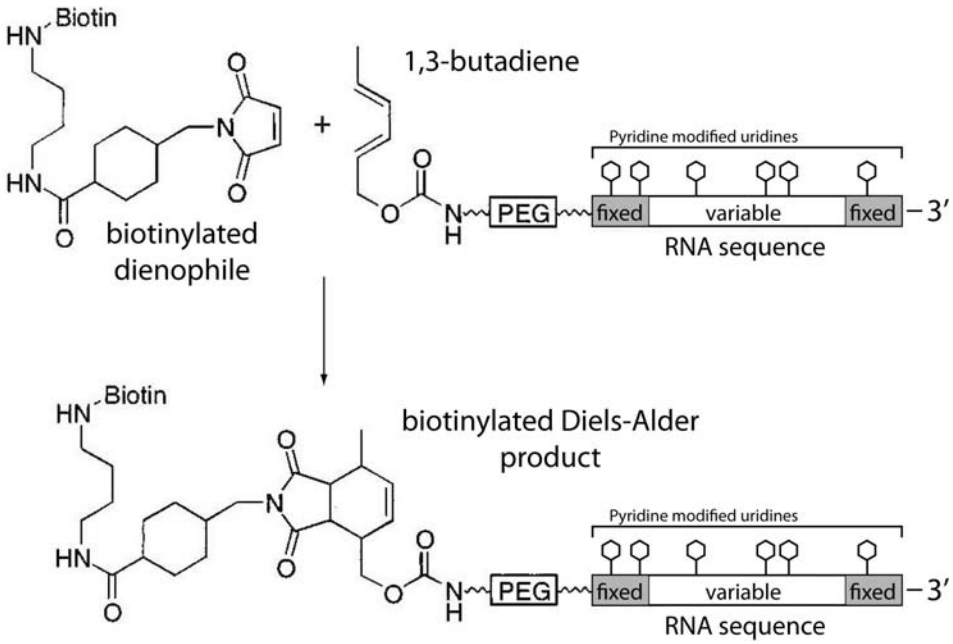



**Fig. (2).** Schematic of the reaction for selection of an RNA with Diels-Alder catalytic potential [19]. PEG stands for the polyethylene glycol linker. In this selection reaction, the pyridine-modified RNA becomes irreversibly bound to the dienophile substrate by a carbon-carbon bond.

it was a single step reaction in which the RNA was permanently modified, which is not truly catalytic according to the definition [20]. Although his lab's discovery was seminal, it was received with skepticism from enzymologists and the scientific community at large. Cech acknowledged this shortcoming and after four long years finally quelled his critics and redeemed his hypothesis by demonstrating that the self-splicing RNA could indeed perform multiple rounds of catalysis [26, 31]. Three years after this proof, he received a phone call from the Nobel Prize committee. The independent work of both Cech and Altman, who shared the 1989 Nobel Prize by showing that the RNA component of RNase P was the catalytic subunit [27, 32], revolutionized the field of biology, ousting the long-held dogma that only proteins could be enzymes. New ideas and disciplines developed around these findings, including the "RNA world" hypothesis and an era of discovering and characterizing new ribozymes. Thus, with regard to the 1997 report by Tarasow *et al.*, it may have been believed that although the authors had not outright demonstrated a catalytic ribozyme, they were warranted in invoking RNA catalysis and the discovery of a Diels-Alderase. Perhaps the optimism and spirit of the time led to the assumption that it would only be a matter of time, possibly their next publication, before the authors would demonstrate enzymatic properties.

The push for discovery of catalysis by artificial RNAs can be seen as an extension of Cech's founding work and a next step in the development of the general field of RNA catalysis. By 1997 Cech's original dilemma, suggesting catalysis without demonstrating an unmodified catalyst or turnover, was considered a paradigm for the future success of a field of research. Indeed, a very early work also published in *Nature* in 1990, where the authors mutated Cech's self-splicing intron and selected variants that could cleave DNA, helped set this precedent [33]. They used the term 'ribozyme' to describe their new RNA despite the fact that they did not demonstrate turnover or catalyst regeneration. The RNA became fused to the DNA substrate during the reaction, thereby inactivating it, but the authors, and apparently the editors and reviewers as well, overlooked this inconsistency. The assumption must have been that future studies would reveal catalytic processes of the same type of chemistry, analogous to Cech's demonstration for the original ribozyme [31]. However, the authors of the 1997 *Nature* paper implied that they had discovered a new catalytic activity, carbon-carbon bond formation, which no ribozyme had ever exhibited previously. However, in the intervening years, no one has ever demonstrated true catalysis with the sequence and composition reported in Tarasow *et al.*, or any of the related sequences. Unlike Cech's case, the dilemma was never resolved for the DAase. In all of their subsequent papers in this field they repeated the same type of assay with a tethered diene reactant, although they became more definitive in their use of language, including referring to the RNA as a Diels-Alderase ribozyme [34-36].

## DEMONSTRATION OF RNA CATALYSIS OF THE DIELS-ALDER REACTION

The discovery of novel catalysts in the field of RNA (ribozymes) is based on a process known as SELEX. In SELEX one starts with a library of a large number (hundreds of billions) of RNAs. RNA has four different molecule building blocks, known as nucleotides. A sequence consisting of 40 nucleotides has $4^{40}$, or approximately $10^{24}$ different possible sequences. In fact, this number is so large that all of the sequences cannot even be made in realistic experiments, and the actual number of sequences is estimate to be $10^{14}$. Thus, the number of possible starting sequences is limited by synthetic technology rather than statistics. Prior to 1994 SELEX had been applied to discover RNA sequences that can bind

other molecules, but not to catalysts. By 1997, research groups had begun the search for new catalysts using SELEX. As often occurs in scientific discovery, there were two groups studying the acceleration of Diels-Alder reactions with RNA at the time of the 1997 *Nature* publication. In 1999, two years after the publication of the *Nature* paper, Jaschke and co-workers used SELEX to discover an RNA catalyst of the Diels-Alder reaction [37]. This RNA catalyst did not require the pyridine modification of uridine, or copper ions, both of which were noted as essential for the RNA described in the 1997 *Nature* paper. The RNA from the Jaschke group also functioned enzymatically with Diels-Alder substrates free in solution, and was later fully characterized using enzyme kinetics, X-ray crystallography and nuclear magnetic resonance (NMR), among other techniques [38-42].

The equations for the analysis of the enzymatic catalysis of the Diels-Alder reaction by RNA were presented by the Jaschke group [43]. The two substrates are known as the dienophile, $S_1$, and the diene, $S_2$ (Part A of Scheme **1**). Since there are two substrates for the Diels-Alder reaction (Part A of Scheme **1**), the catalytic rate, v, is expressed using the Michaelis-Menten equations for both $S_1$ and $S_2$:

$$v = \frac{v_{app}[S_1]}{K_{m,1} + [S_1]}$$
$$v_{app} = \frac{v_{\max}[S_2]}{K_{m,2} + [S_2]} \quad (1)$$

where $K_{m,1}$ and $K_{m,2}$ are the Michaelis constants for substrates 1 and 2, respectively. In a catalytic process, thousands of substrate molecules of substrate, $S_1$ and $S_2$, react to form product in the presence of each molecule of catalyst. The rate increases as the substrate concentrations, $[S_1]$ and $[S_2]$ increase, but the rate levels off at high substrate concentration, $[S_1] >> K_{m,1}$ and $[S_2] >> K_{m,2}$. The reason for this behavior is that the rate is limited by diffusion of molecules in solution when the substrate concentrations are low. However, at high concentration the substrates are essentially always bound to the enzyme and the rate is limited by the intrinsic ability of the enzyme to form the bonds and release the product. These features of the kinetic model are essential for any description of enzymatic catalysis by proteins, and the kinetic analysis is equally valid for any true RNA catalyst.

In the experiments reported in the 1997 *Nature* paper and in all of the subsequent work by these authors using the modified RNAs [19, 34-36], the diene, $S_2$, is chemically attached to the RNA while only the concentration of the dienophile, $S_1$, can be varied (Part B of Scheme **1**). Therefore, it is not possible to independently vary both substrate concentrations, $S_1$ and $S_2$, and therefore not possible to determine Michaelis-Menten kinetics of a two-substrate reaction (Eqn. 1 and part A of Scheme **1**). This means that enzymatic turnover is not possible. The catalyst is not regenerated because the reaction is a second-order reaction with suicide inhibition of the enzyme (part B of Scheme **1**). It is important to distinguish the work in the 1997 *Nature* paper from studies too numerous to cite here that have demonstrated the catalytic potential of RNA for the Diels-Alder reaction [38-42] and a range of other chemical reactions. At issue here is not whether RNA can catalyze a range of reactions, but whether an early account of RNA catalysis was overstated and misleading.

The authors of the 1997 *Nature* paper employed 'RNA-catalysed' as a key word in their title. In addition, the article connected well with two other very popular topics, SELEX and catalysis of carbon-carbon bond formation. SELEX was still relatively new and identification of a novel RNA binding molecule alone could be rewarded with a respectable publication [24, 44]. Likewise robust, stereo-controlled carbon-carbon bond formation has always been a need in synthetic chemistry [28]. Any molecule that can catalyze such a reaction is very attractive. The SELEX technique and demonstration of carbon-carbon bond formation appear to be sound experimental approaches. However, one might doubt that this work alone would have been sufficient for publication in the prestigious *Nature* journal, if the authors had simply reported Diels-Alder carbon-carbon bond formation in a bimolecular reaction aided by the presence of conjugated RNA [10]. As we shall see, the article was implicitly built around the assumption that the RNA was an enzyme, subtly exploiting the exciting popularity of ribozymes. Unfortunately, neither the data presented nor subsequent work support the statements made in the paper. In light of our observations we now discuss critical aspects of the paper that break down under scrutiny for the root cause of improperly treating the RNA as an enzyme. The following two sections are a technical examination of the facts and may be skipped by the more general reader.

## THE DESIGN AND SELECTION OF A 'CATALYTIC' RNA

The RNA was designed to i) be 146 nucleotides long with a variable central region and DNA ends of a fixed sequence, ii) contain uridine nucleotides modified at the 5-position with a pyridine moiety, and iii) be covalently bonded to a Diels-Alder substrate (the diene) through a flexible linker (Fig. **2**) [19]. The variable region was synthesized chemically such that it consisted of a random sequence of the four nucleotides A, G, C and U*, where U* represents pyridine-modified uridine. The selection implemented required a chemical reaction, called the Diels-Alder reaction, between the attached diene and a biotinylated dienophile in solution. When the RNA-diene conjugate reacted with the biotin-dienophile substrate it was detected by binding of streptavidin, a protein that specifically recognizes biotin. The streptavidin-RNA complex was then separated from unreacted components on a native polyacrylamide gel. The complex of reacted RNA and streptavidin was excised from the gel and the RNA sequence subjected to several more rounds of amplification and selection. The selection process was not unlike others described in the literature [24]. Indeed, the use of conjugated and biotinylated substrates was a clever application. Despite these innovations, the approach inherently selected for a single chemical reaction to form a carbon-carbon bond, not a multiple-turnover enzymatic process. To actually demonstrate enzymatic catalysis, as implied throughout the article, the initial lead RNAs from these selections would need to undergo further optimization to show turnover. The authors instead chose to focus on one of the first generation RNAs, called DA22, that exhibited the best

acceleration by using analyses designed for enzyme characterization.

## EMPLOYING THE MICHAELIS-MENTEN EQUATION TO IMPLY ENZYME KINETICS

Although the actual word enzyme is never used in the 1997 *Nature* paper, subsequent papers by these authors on the bimolecular reaction refer to DA22 as a Diels-Alderase (DAase) and later call the sequence an enzyme [19, 34-36, 45]. Despite this fact, the authors treat the observed rate of formation of a product using Michaelis-Menten enzyme kinetics. Of course, the application of an enzyme kinetic scheme to a bimolecular reaction is incorrect in the strict sense, and therefore the enzyme kinetic analysis presented in the *Nature* paper is erroneous. In order to understand why, we examine how their analysis was done.

To contrive an estimate for the rate acceleration for the reaction, as would be expected for any catalyst, the authors needed to generate an apparent second-order rate constant for the 'catalyzed' reaction, $k_{cat}$, and compare it to the second-order rate constant they actually observed for the uncatalyzed reaction, $k_{uncat}$. In the Michaelis-Menten theory $v_{max} = k_{cat}[E]$, which implies that one can vary the enzyme concentration, [E], separately from the substrate concentration. Since that was not possible when one of their substrates was chemically tethered to the "enzyme", the authors of the 1997 Nature paper measured an observed pseudo-first order rate constant $k_{obs}$, which has an apparent catalytic rate constant $k_{cat}^{app}$. This approach effectively eliminates the enzyme from the analysis.

Instead of analyzing the Michaelis-Menten curve directly, the authors produced and analyzed a Lineweaver-Burke plot and extracted separate values for $k_{cat}^{app}$ and $K_m$. A Lineweaver-Burke plot is a double reciprocal analysis used to linearize the Michaelis-Menten equation.

$$\frac{1}{v} = \frac{K_m}{v_{max}}\frac{1}{[S]} + \frac{1}{v_{max}} \qquad (2)$$

or using the pseudo-first order approximation symbols of the 1997 *Nature* paper,

$$\frac{1}{v} = \frac{K_m}{k_{cat}^{app}}\frac{1}{[S]} + \frac{1}{k_{cat}^{app}} \qquad (3)$$

where S is implicitly $S_1$, the dienophile. In the Lineweaver-Burke analysis in Eqn. 3 the ratio $K_m/k_{cat}^{app}$ is the slope and $1/k_{cat}^{app}$ is the intercept of a line. The assumption of Lineweaver-Burke is that the data extend to the point where saturation occurs, where $[S] > K_m$. Before the advent of the personal computer with programs for routine fitting using non-linear least squares, the linearization of non-linear data was a key step in analysis. However, in the present case, the range of the data is sufficiently small that the data are linear already (see Fig. **3** of the 1997 *Nature* paper and the inset of Fig. **3** in this paper). Consequently, several problems arise in the analysis based on their assumptions and improper use of equations and data fitting.

Using slope and intercept of the Lineweaver-Burke plot (Eqn. 3), the authors of the 1997 *Nature* paper obtained Michaelis-Menten parameters, assumed that $k_{cat}^{app} = k_{cat}$, and concluded that the ratio of $k_{cat}/k_{uncat}$ corresponded to an 800-fold acceleration of the reaction. To properly determine $K_m$, the authors would have needed to fit their data to a Michaelis-Menten plot of reaction rate versus substrate concentration, $k_{cat}^{app}$ vs. [S] up to the point where the curve begins to saturate at least for the dienophile that is free in solution (i.e. $S = S_1$). However, the substrate, $S_1$, is sufficiently insoluble that the data never surpass the linear range and cannot be accurately fit to a Michaelis-Menten plot (Fig. **3**). $K_m$ is defined as the substrate concentration at which 1/2 $V_{max}$ is reached, which is shown in the relevant form as 1/2 $k_{cat}^{app}$ in Fig. (**3**). The statistical inaccuracy of the method becomes large when $[S]_{max} << K_m$, which is certainly the case here as shown in the inset to Fig. (**3**). Despite the limited range of data and erroneous nature of the analysis, one can use non-linear regression using the program IgorPro5.0 (Wavemetrics) to fit the data to the Michaelis-Menten kinetic model to obtain parameters $k_{cat}^{app} = 0.064\ s^{-1}$ and $K_m = 16$ mM (see Appendix). A range of acceptable fits is given by the estimate of the standard deviation for the non-linear squares regression shown by the calculated lower and upper bound curves given by the dashed lines in Fig. (**3**).

The data in Fig. (**3**) of the 1997 *Nature* paper [19] were fit to a linear function ($k_{obs}$ vs. [S]). Hence, there is no basis for unique determination of separate values for $k_{cat}^{app}$ and $K_m$ using a Lineweaver-Burke analysis, which involves taking the reciprocal of the data and fitting $1/k_{obs}$ vs. 1/[S]. Consequently, the values of $k_{cat}^{app} = 0.011\ s^{-1}$ and $K_m = 2.3$ mM reported in the 1997 *Nature* paper would be in error by a factor of ~7 and ~6 in $k_{cat}^{app}$ and $K_m$, respectively. The comparison serves to illustrate complications that arise when data do not conform to the assumptions of the model used for fitting. $K_m$ has no interpretation here since there is no reversible enzyme-substrate complex and the catalytic rate constant has no meaning since the experiment irreversibly alters the "catalyst". For example, at the highest substrate concentration of 500 μM, at most only 0.1% of the "substrate" could possibly react since its reaction partner, the "enzyme" tethered to the diene shown in Fig. (**2**), is present at 1000-fold lower concentration. The quantitative values presented have no significance and the fit in Fig. (**3**) simply serves to illustrate the inconsistency in the model as applied.

## REPERCUSSIONS OF MAXIMIZING IMPACT BY DISTORTION OF SCIENTIFIC PHRASES

In the case of the 1997 *Nature* paper, the authors discovered an RNA that had "catalytic potential." The application of a scientific description only appropriate for enzymes gives the reader the impression that the catalytic potential of this system was realized in their work. However, the Michaelis-Menten formalism and the Lineweaver-Burke treatment were applied incorrectly. The system is not catalytic and there is no evidence for specificity of the binding interactions of the substrate or product. The catalytic potential of this system was never realized in further work, perhaps because of the insolubility of the reagents. The language choice in the 1997 *Nature* paper served to appeal to fields of research in RNA catalysis, the origin of life in an RNA world, and a biotechnology community that seeks to design practical enzymes capable of useful synthetic transformations. Certain aspects of the report, such as the obligatory requirement for pyridine and $Cu^{2+}$, are in doubt based on subsequent work [37, 46]. The writing style in this and subsequent publications leads

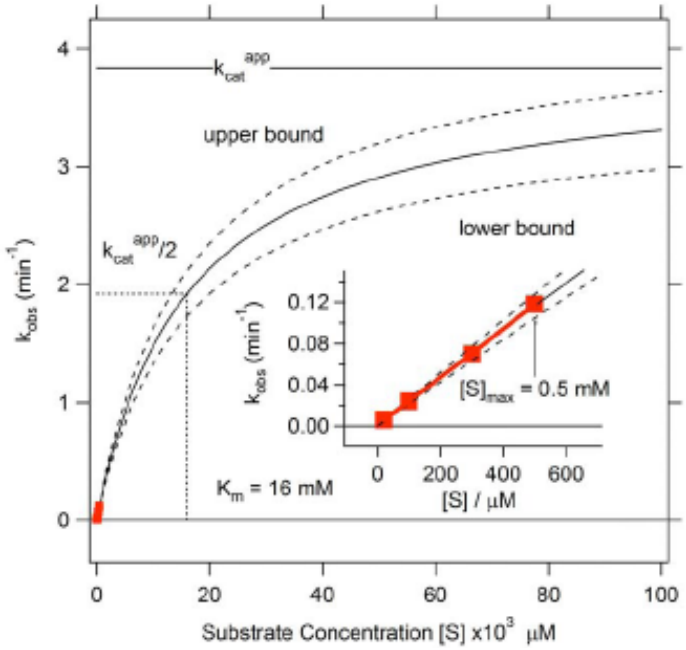



**Fig. (3).** Fit of the data from Fig. (**3**) of the 1997 *Nature* paper combined with a model for Michaelis-Menten kinetics. The data are shown as red squares. The inset has approximately the limits shown in the 1997 *Nature* paper. The full plot shows how poorly the data sample the full Michaelis-Menten curve. The data can indeed be fit to a linear model (red line) and the Michaelis-Menten curve is also linear over the range of the data. The dashed lines indicate 10% deviations of the ratio $k_{cat}^{app}/K_m$.

the reader to believe that the processes described are applicable in their current form, not that there are significant technical hurdles that must still be overcome. The use of enzyme kinetic analysis disguises the fact that the process they describe is a second-order cycloaddition reaction on the picomolar scale isolated from a gel, not a scalable catalytic process. Focusing undue attention on the assumption that the RNA was a "ribozyme" only served to further obscure any quality work in the 1997 *Nature* paper.

Science has become a business and as such requires scientists, the small business owners, to promote their research and compete for resources in a free-market economy. The promotion of research is generally secondary, as it should be, to honest research and reporting. Yet the drive to be noticed in science and obtain prestige and funding, or more often simply to survive, can constantly tempt even the most moral and resolute scientist. Few scientists would admit the occasional temptation to improve impact by catering their data to tell a story that capitalizes on a vogue topic or well-crafted manuscript title [9, 10]. Ironically, these are the stories journals seem most eager to publish, raising the question of who is really to blame [10]. To ensure a simple ethics code is maintained we recommend that authors i) evaluate the impact of their research based solely on the merits of the results, ii) use precise and conservative scientific language to tell their story of discovery and iii) make sure their use of language is qualified based upon the results of their experiments, not current trends in the literature, scientific community or popular media. We believe that promoting discovery within these guidelines will help avoid most ethical issues surrounding scientific language.

Scientists are expected to uphold the highest standards of moral conduct. This not only includes performing experiments and handling of patient data, but also in the reporting of results. Abusing scientific publication with buzzwords or chic phrases to improve impact can inflate the value of research, much like the inflation of currency in an economy. It also degrades trust among colleagues, who may seek to replicate or build upon the results of poorly presented publications. Progress in a field can be slowed by the misallocation of resources. Repeated violations do not go unnoticed by the research community and, if flagrant or often enough, can attract the attention of the media and general public, reinforcing a mistrust of science. Although the publication we chose as a case study did not previously raise ethical concerns, it serves as an excellent example for precisely that reason. Because the issue of responsible use of scientific language in publication is not well-understood [10], most publications that abuse scientific language in these subtle ways fall below the radar of falsification, fabrication, plagiarism, and redundant or duplicate publication, the most disconcerting ethical concerns in scientific publication [16, 17].

In the modern scientific era of biotechnology and nanotechnology, and all the -omics that follow with it, interdisciplinary research is becoming the norm. For example, the work described in the 1997 *Nature* paper spans molecular biology, chemical biology, enzymology and synthetic chemistry. Thus, it is difficult for any single reviewer to have mastery of all of the experimental methods. For this reason it is all the more imperative that the writing style be a clear narrative that seeks to explain observations rather than advertise results that have the most impact.

## ACKNOWLEDGEMENTS

This study was supported by an NIH Kirschstein Fellowship to K.T.G. (F32HD060377) and the W.M. Keck Foundation.

## APPENDIX: SUBROUTINE FOR CALCULATION OF MICHAELIS-MENTEN PARAMETERS

The application of a subroutine of this type involves pasting the text into the **Procedure Window** of the Wavemetrics program. The program recognizes this as a fitting function and it automatically appears on the pull-down **Analysis** menu item. Similar implementation is possible in a range of commonly used software.

```
Function michaelis_menten(w,s)

Wave w; Variable s

Variable vmax, km, s

vmax = w[0]

km = w[1]

v = vmax*s/(km+s)

return v

End
```

## REFERENCES


[1] Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995; 5235: 467-70.

[2] Schena M, Heller RA, Theriault TP, Konrad K, Lachenmeier E, Davis RW. Microarrays: biotechnology's discovery platform for functional genomics. Trends Biotech 1998; 16: 301-6.

[3] Shalon D, Smith SJ, Brown PO. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. Genome Res 1996; 6: 639-45.

[4] Brenner S, Johnson M, Bridgham J, *et al*. Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat Biotech 2000; 18: 630-4.

[5] Margulies M, Egholm M, Altman WE, *et al*. Genome sequencing in microfabricated high-density picolitre reactors. Nature 2005; 437: 376-80.

[6] Barski A, Cuddapah S, Cui K, *et al*. High-Resolution Profiling of Histone Methylations in the Human Genome. Cell 2007; 129: 823-37.

[7] Fox JL. Researchers discuss NIH's nanotechnology initiative. Nat Biotechnol 2000; 18: 821.

[8] David A. Write a classic paper. Br Med J 1990; 300: 30-1.

[9] Lawrence PA. Science or alchemy? Nat Rev Genet 2001; 2: 139-42.

[10] Lawrence PA. The politics of publication. Nature 2003; 422: 259-61.

[11] Marks AR. The economy of science. J Clin Invest 2004; 114: 871.

[12] Mirowski P. The Effortless Economy of Science? Durham, NC: Duke University Press 2004.

[13] Jiang Y, Jahagirdar BN, Reinhardt RL, *et al*. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41-9.

[14] Van der Heyden MAG, Derks van de Ven T, Opthof T. Fraud and misconduct: the stem cell seduction. Neth Heart J 2009; 17: 25-9.

[15] Vogel G. Falsification Charge Highlights Image-Manipulation Standards. Science 2008; 322: 356.

[16] Benos DJ, Fabres J, Farmer J, *et al*. Ethics and scientific publication. Adv Physiol Educ 2005; 29: 59-74.

[17] Graf C, Wager E, Bowman A, Fiack S, Scott-Lichter D, Robinson A. Best Practice Guidelines on Publication Ethics: a Publisher's Perspective. Int J Clin Pract 2007; 61: 1-26.

[18] Day RA, Gastel B. How to Write and Publish a Scientific Paper. Santa Barbara, CA: Greenwood Press 2006.

[19] Tarasow TM, Tarasow SL, Eaton BE. RNA-catalysed carbon-carbon bond formation. Nature 1997; 389: 54-7.

[20] Kruger K, Grabowski PJ, Zaug AJ, Sands J, Gottschling DE, Cech TR. Self-Splicing RNA – Auto-Excision and Auto-Cyclization of the Ribosomal-RNA Intervening Sequence of Tetrahymena. Cell 1982; 31: 147-57.

[21] Joyce GF. Amplification, mutation and selection of catalytic RNA. Gene 1989; 82: 83-7.

[22] Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 1990; 4968: 505-10.

[23] Beaudry AA, Joyce GF. Directed evolution of an RNA enzyme. Science 1992; 5070: 635-41.

[24] Tuerk C. Using the SELEX combinatorial chemistry process to find high affinity nucleic acid ligands to target molecules. Meth Mol Biol 1997; 67: 219-30.

[25] Waldrop MM. Catalytic RNA wins chemistry Nobel. Science 1989; 246: 325.

[26] Cech TR. Nobel lecture. Self-splicing and enzymatic activity of an intervening sequence RNA from Tetrahymena. Biosci Rep 1990; 10: 239-61.

[27] Altman S. Nobel lecture. Enzymatic cleavage of RNA by RNA. Biosci Rep 1990; 10: 317-37.

[28] Corey EJ, Cheng X. The Logic of Chemical Synthesis. New York, NY: Wiley Press 1989.

[29] Hilvert D. Antibody catalysis of carbon-carbon bond formation. Ciba Found Symp 1991; 159: 174-83.

[30] Morris KN, Tarasow TM, Julin CM, Simons SL, Hilvert D, Gold L. Enrichment for RNA molecules that bind a Diels-Alder transition state analog. Proc Natl Acad Sci USA 1994; 91: 13028-32.

[31] Zaug AJ, Cech TR. The intervening sequence RNA of Tetrahymena is an enzyme. Science 1986; 231: 470-5.

[32] Guerrier-Takada C, Gardiner K, Marsh T, Pace N, Altman S. The RNA Moiety of Ribonuclease-P Is the Catalytic Subunit of the Enzyme. Cell 1983; 35: 849-57.

[33] Robertson DL, Joyce GF. Selection *in vitro* of an RNA enzyme that specifically cleaves single-stranded DNA. Nature 1990; 344: 467-8.

[34] Tarasow TM, Tarasow SL, Tu C, Kellogg E, Eaton BE. Characteristics of an RNA Diels-Alderase active site. J Am Chem Soc 1999; 121: 3614-7.

[35] Tarasow TM, Tarasow SL, Eaton BE. RNA Diels-Alderases: relationships between unique sequences and catalytic function. J Am Chem Soc 2000; 122: 1015-21.

[36] Tarasow TM, Kellogg E, Holley BL, Nieuwlandt D, Tarasow SL, Eaton BE. The effect of mutation on RNA Diels-Alderases. J Am Chem Soc 2004; 126: 11843-51.

[37] Seelig B, Jaschke A. A small catalytic RNA motif with Diels-Alderase activity. Chem Biol 1999; 6: 167-76.

[38] Seelig B, Keiper S, Stuhlmann F, Jaschke A. Enantioselective ribozyme catalysis of a bimolecular cycloaddition reaction. Angew Chem Int Ed 2000; 39: 4576-9.

[39] Stuhlmann F, Jaschke A. Characterization of an RNA active site: interactions between a Diels-Alderase ribozyme and its substrates and products. J Am Chem Soc 2002; 124: 3238-44.

[40] Keiper S, Bebenroth D, Seelig B, Westhof E, Jaschke A. Architecture of a Diels-Alderase ribozyme with a preformed catalytic pocket. Chem Biol 2004; 11: 1217-27.

[41] Serganov A, Keiper S, Malinina L, *et al*. Structural basis for Diels-Alder ribozyme-catalyzed carbon-carbon bond formation. Nat Struct Mol Biol 2005; 12: 218-24.

[42] Manoharan V, Furtig B, Jaschke A, Schwalbe H. Metal-induced folding of Diels-Alderase ribozymes studied by static and time-resolved NMR spectroscopy. J Am Chem Soc 2009; 131: 6261-70.

[43] Agresti JJ, Kelly BT, Jaschke A, Griffiths AD. Selection of ribozymes that catalyse multiple-turnover Diels-Alder cycloadditions by using *in vitro* compartmentalization. Proc Natl Acad Sci USA 2005; 102: 16170-5.

[44] Klug SJ, Famulok M. All you wanted to know about SELEX. Mol Biol Rep 1994; 20: 97-107.

[45] Tarasow TM, Eaton BE. Dressed for Success: Realizing the Catalytic Potential of RNA. Biopolymers 1998; 48: 29-37.

[46] Gagnon KT, Ju S-Y, Goshe M, Maxwell ES, Franzen S. A role for hydrophobicity in a Diels-Alder reaction catalyzed by pyridyl-modified RNA. Nucleic Acids Res 2009; 37: 3074-82.

Received: September 23, 2010 Revised: October 28, 2010 Accepted: November 04, 2010