

# Journal of Unsolved Questions

# Walls and JunQtions Communicating Science

Communicating Math to the Masses Prof. Cedric Villani

> Measuring Science in Germany Prof. Stefan Hornbostel

The Anatomy of the Wall The Falling Walls Conference 2011

ISSN 2192-0745 http://junq.info/

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# Preface

## **Editorial Note**

## Dear Reader,

It is my pleasure to present to you the third issue of the Journal of Unsolved Questions, with the topic "Communicating Science", which is, of course, a topic close to our hearts. JunQ is most and foremost a tool for communicating science, successful or not, either to other scientists or to the non-scientific public. I know that often a scientific article can look appalling for someone outside of the area, but it does not always have to be like that. We can put aside the mystic scientific details and focus on the essence of work: why did they do this? Does it bring anything new and useful for the world? Does it answer any essential pertinent question (scientific or not)? Scientists can only profit from a non-scientific peer review, as the people outside of the scientific community can give a different perspective regarding the impact of the work or regarding the needs of the real world when it comes to new research topics. But the scientific community must also take the other side into consideration. What does the scientific community do to get closer to the public? Probably the most common answer will be "not much". In fact few scientists go through the effort to occasionally simplify their language and divulge their work to the non-scientific crowd. We all know the scientist stereotype, totally absent of any social and communicating skills, trapped in a lab with his lab coat and colorful potions. Thanks to the new media, social networks, and the increase in "popular science" magazines, this panorama is changing at a good pace. In this issue we pay particular attention to a German initiative in science communication, the Falling Walls Conference, which is an annual global gathering of individuals from more than 75 countries. In each edition, 20 of the world's leading scientists are invited to present their current research. The conferences have been held since 2009 on the anniversary of the fall of the Berlin Wall to celebrate this historic day and give a fresh look to the world of tomorrow. The JUnQ team was present in this year's edition where we also made a live Twitter coverage of the most interesting talks during the conference. (Follow us on Twitter: @JUnQJournal) You can find a summary of the conference's most important topics in the article "When Walls Come Down, Inspiration is Scattered Everywhere ". It was possible to change a few words with the renowned mathematician Cédric Villani, whose opinion about "Communicating Math to the Masses" is printed in this very issue, additionally editorial board member Thomas Jagau gives his opinion about the phrase "sustainable chemistry" in his essay "Can Chemistry be Green?".

This passing year has been very successful in gathering contributions for JunQ. Approximately 20 contributions were sent to us from all over the world, they were carefully reviewed and selected. Four of these contributions covering a plethora of scientific disciplines can be found in this issue. This year has also been important for the JUnQ team in terms of collaborations with other institutions. Since July we have been working together with the German speaking broadcasting station detector.fm. This includes a monthly podcast, "Question of the Month", about an unsolved question in science. You can access the contributions either at our website (http://junq.info) or at http://detektor.fm.

Spreading JUnQ's message was facilitated by the many invitations we got to comment on the importance of negative and null-results and the idea of JUnQ in the media. To name one example, an article about JunQ was published in Times Higher Education entitled "Gems in the research scrapheap".<sup>1</sup> Furthermore the editorial board was invited to write the editorial article in the magazine "Nachrichten aus der Chemie" published by the Gesellschaft deutscher Chemiker.<sup>2</sup> Something we are very excited about is the formation of an association (German, Verein) in conjunction with the JunQ project. The bureaucratic process is still in progress, but this will hopefully allow us to organize our actions and collaborations better and will help in making the Journal of Unsolved Questions the tool for communication we have aimed at since our first issue. But that is impossible without your help, as usual we ask readers to participate actively in this journal either by taking part in our organized talks, leaving a comment on our website or, most importantly, sending us your contributions. We are waiting for you!

- Rute André

<sup>&</sup>lt;sup>1</sup>http://www.timeshighereducation.co.uk/story.asp?sectioncode=26&storycode=417276&c=1

<sup>&</sup>lt;sup>2</sup>Nachrichten aus der Chemie, September 2011, page 807

## **Measuring Science in Germany**

Interview with Professor Stefan Hornbostel



What are the strengths and weaknesses of the German scientific system? German science has helped to resolve many questions, but has long been unable to assess its own performance. To fill this conspicuous hole, the *Deutsche Forschungsgemeinschaft* established the Institute for Research Evaluation and Quality Assurance (iFQ) in 2005 and appointed Stefan Hornbostel as founding director. A sociologist by training, he currently holds a professorship for research evaluation at the Humboldt University in Berlin as well.

During his visit to Mainz in June 2011, we talked to Prof. Hornbostel about his work at the iFQ, the German scientific system, and the changes it underwent in recent years.

**JUnQ:** Professor Hornbostel, you are the director of the Institute for Research Information and Quality Assurance (iFQ - *Institut für Forschungsinformation und Qualitätssicherung*). What is the purpose of this institute? **Hornbostel:** Originally, the iFQ was the reply to an evaluation of the Max Planck Society (MPG – *Max-Planck-Gesellschaft*) and the German Research Foundation (DFG – *Deutsche Forschungsgemeinschaft*): International reviewers had the impression that we barely know how the German scientific system works, what its incentives and deficits are, and what impact funding programs and political actions have. The purpose of the iFQ is to shed light on these questions.

**JUnQ:** Are there institutions in other countries that served as a model for the iFQ?

**Hornbostel:** Yes, there are. Germany is a latecomer in that regard. In the UK and the US, but also in the Netherlands, "Science of Science" arose much earlier. Its tradition goes back to the 1920s and 1930s. Yet in Germany, the idea was of little importance in the following years, while it was pursued more systematically in the US. In Germany, a lot of single disciplines like history of science, philosophy of science, and sociology of science are involved in this field, but an integrated approach that also includes empirical methods is relatively new in Germany.

JUnQ: Can you outline what methods you use?

**Hornbostel:** We mostly use standard methods from social sciences like surveys, interviews, and text analyses. But some tools are new and were specifically developed for our field, for example bibliometrics or the analysis of funding programs.

**JUnQ:** Do you target all disciplines, i.e. natural sciences, social sciences, and the humanities?

**Hornbostel**: Yes, we do. This is related to the fact that, when it was founded, the iFQ was strongly embedded in the DFG. From an international perspective, it is rather an ex-

ception that one single institution – the DFG – is in charge of funding all disciplines. Most countries distinguish at least between large scientific fields, but since there is this special German tradition, we deal with all disciplines, including the humanities. Not every method can be applied to all areas due to different scientific cultures, however, we do not want to restrict our studies to natural sciences, but we try to examine all disciplines.

**JUnQ:** Many scientists are sceptical towards evaluations in general as they consider them to be time-consuming and useless. Have you encountered such objections? How do you cope with them?

**Hornbostel:** This objection is not only raised by natural scientists, but also in the humanities. The introduction of quality and performance measures in science and the public discourse about it has led to enormous changes in the German scientific system, though. As evaluations are nowadays carried out in a routine manner, we should legitimately ask: Is the effort still in due proportion to its benefit? It may well be that evaluation efforts can be reduced once a certain level of performance is reached without lowering the results' quality. Our methods need to evolve in coevolution with their target areas, they need to be flexible so that they can be adapted to changes in science.

**JUnQ:** The question is often raised whether it is possible at all to "measure" science. Do you think this is possible?

**Hornbostel:** We can refer to a great example. Derek John de Solla Price, one of the founding fathers of bibliometrics, once began a lecture with the question: Why should we not apply scientific methods to science itself? This is an obvious question: Why should the sector that is most involved in the generation of methodologically controlled knowledge exclude itself from its studies? We also need to bear in mind that science is nowadays in completely different shape than it was one hundred years ago. The amount of results, the cost, and the complexity of scientific infrastruc-

tures has dramatically increased. It is simply necessary to think about a meaningful concept how to operationalize and measure important quantities like advancement of knowledge but also efficiency.

**JUnQ:** In science, quantities like the *h*-Index and the Journal Impact Factor are often used to assess scientific performance. What do you think of these numbers?

**Hornbostel:** I have a low opinion of the *h*-Index. This index tries to condense several pieces of information that were previously represented by different indices. Such attempts to form a "super-index" usually cause more problems than benefits. The *h*-Index suffers from various problems that may lead to misjudgments if one is not aware that other factors affect it, apart from scientific performance. The Journal Impact Factor was invented to help librarians with the decision what journals to subscribe or unsubscribe to. While it served that purpose well, it is nowadays misused to assess individual scientists' work, which cannot be justified at all as there is a high danger of wrong conclusions. In short, this indicator is helpful for certain application fields, but it is also misused, thereby leading to problematic results.

**JUnQ:** The iFQ also surveys scientists about their satisfaction with their personal situation and the shape, in which science is. Can you outline how the German scientific community perceives itself?

**Hornbostel:** We just completed an extensive survey among German professors, which included several issues of current science policy. I can summarize the results roughly as follows: German scientists from nearly all disciplines consider themselves to be competitive on an international level and a majority thinks that the funding conditions for research projects are very good. German scientists are in general not averse to rivalry and competition for public attention, money, and publications. However, we noticed a certain weariness concerning the numerous application-based funding procedures. Throughout all disciplines, scientists criticize the increasing scarcity of regular funding.

JUnQ: Has this perception changed over the years?

**Hornbostel:** This is not easy to determine as there were not many systematic surveys in earlier years. Fortunately, the Allensbach Institute conducted surveys among German faculty members in the 1970s and 1980s. In comparison to these data, we clearly observed that the profound changes in the German scientific system since the beginning of the 1990s have left their marks in the scientists' perceptions. For example, we noticed a shift in perception regarding the question if money always accumulates in the hands of the same people. In this respect, scientists are now more critical than they were thirty years ago. Furthermore, today's faculty members assign a more important role to impression management than their colleagues did in the 1970s.

JUnQ: What caused these changes?

**Hornbostel:** It is obvious that they result from an increasing focus on performance and competition in the German scientific system. Since the 1970s, the gap between regular funding and external funding has widened significantly. Today, it is almost impossible to do research without external application-based funding, while in the 1970s the level of regular funding was significantly higher. In addition, the performance of today's scientists is constantly monitored on many levels: By the universities, by the federal states, by the German Council of Science and Humanities (*Wissenschaftsrat*), and by external rankings. A modern scientist's life is different than it was in the past and the focus on competition is much stronger.

**JUnQ:** A key issue in the public debate regarding competition in science is the Excellence Initiative.

Hornbostel: The iFQ is evaluating the Excellence Initiative and has collected a lot of data on this issue. The picture we get is a little paradoxical. On the one hand, the Excellence Initiative was one of the great endeavors in recent years. In many interviews at different universities, we always hear the same message that things got going, not only due to additional money, but because of the novel idea to compete for prestigious titles that are perceived by the public. Outdated structures were abandoned, new structures emerged that often crossed sectional boundaries, and many novel concepts were given a try. In short, the Excellence Initiative generated momentum in the German universities. On the other hand, we surveyed professors on the question whether this concept of funding is suited to push research in Germany forward. In this regard, the impression is rather negative. In almost no scientific field, the Excellence Initiative is considered to be a promising funding concept. Maybe, the truth lies in the middle. That is, the Excellence Initiative is unsuitable as a permanent institution, but in its historical setting, it was important and helpful. This ranges from interdisciplinary contacts and new forms of organization to enhanced recruiting procedures and novel forms of support for young scientists. For example, graduate schools have emerged. Such new concepts did not only lead to changes in very short time periods, but they also created enthusiasm for experiments. This unideological eagerness for experiment is something new to the German scientific system and something very positive.

JUnQ: Professor Hornbostel, I thank you for the interview.

- Thomas Jagau

# When Walls Come Down, Inspiration Is Scattered Everywhere. The Falling Walls Conference Berlin 2011

The symbolic yet sturdy walls between scientific disciplines and paradigms still often hinder joint efforts and integrated approaches in science, which are needed to face walls in the form of current and future societal, environmental and economic challenges. The Falling Walls Conference, a scientific mega-event that is held annually in Berlin on the historic date the Berlin Wall came down, attempts to tear down these walls. It assembles twenty leading international scientists from diverse areas of research and a good seven hundred attendees to share knowledge and opinions about 'which are the next walls to fall' and how to bring them down.

## The Format

What started as an experiment in 2009 has since become a tradition, a fairly "normal event" as initiator of the conference Sebastian Turner (under-)stated in his opening address, characterizing Falling Walls as a casual conversation rather than a conference, a place where academics talk to the audience like to a friend. The conference is organized by the Falling Walls Foundation, a non-profit organization founded in support of science and humanities by the Berlin Senate for Education, Science and Research and directed by Turner. Its list of supporters reads like a veritable who is who of the German scientific landscape, among them the German Ministry for Education and Research, and leading research institutions like the Fraunhofer and the Max Planck Society, the Helmholtz Association, and the like. Also participating in this distinguished circle of friends' annual chat is Chancellor Angela Merkel, who in her keynote stressed the need for the mutual exchange of ideas to transcend borders on the way to a new Europe.

Each year, Turner and his organization team of just three people bring together "forward thinking individuals" from science, politics, economy, and culture. A lot of work and consideration goes into assembling the crowd on the podium, in front of it, and behind the scenes in the press area. Speakers are required to conduct excellent research in their field and are hand-picked by recommendation of scientific peers. Moreover, breakthrough-appeal of their research is considered pivotal. As in science, some of the most farreaching breakthroughs were accomplished by fairly unknown researchers and off the beaten path (as is well illustrated by Einstein and the theory of relativity); this strategy seems to make sense: "To lure Einstein to come to Berlin, Max Planck traveled to Zürich. Had we asked Max Planck, he could have named Einstein as a candidate for the conference early on. In this way it would have been possible to find him before he established the general theory of relativity and even before he was awarded the Nobel Price." Turner explains. Of course, not everything that deserves to be presented at the conference can be considered, he concedes, as there are enough outstanding researchers to fill one or several weeks, some so productive that they can present relevant advancements each year. The concentration of Nobel laureates and holders of other notable scientific awards at the conference is imposingly high.

As society is becoming an increasingly demanding consumer of both application-oriented and mass medially communicable knowledge, the external view on scientific research becomes more and more important. This is exemplified by the promotional effort scientific events like the Falling Walls Conference take on nowadays. An expert in merchandising almost everything from the Dresdner Frauenkirche to the Federal Republic of Germany (for which he is said to have coined the slogan: land of ideas), Turner knows a good deal about large-scale communication. Fortunately, Turner comments with a wink, when asked how difficult science is to merchandise, 'it is rather easy and takes little funding. This would become even more apparent had science the marketing budgets of washing agents". The next question, of course, is who actually shall and realistically can be reached by the Falling Walls format. The audience of "decision makers" is as well selected as are the speakers; and with high ticket fees, the exclusive look and feel of the event, questions about the walls around this very wall breaking event arise. Since the conference, according to Turner, addresses 'everybody interested in the future", and since one of its declared aims is to "inspire people to break down the walls that we face today", easy access is of importance for the credibility of the event. Asked about possibilities and difficulties in communicating science to the masses, and Falling Walls' role in it, Turner declares that this was indeed a challenge, and sketches the steps the organizers take to approach it: "No obstacle is invincible. The first question is how scientific complexity can be made understandable without distorting it too much. The question of drawing a connection, pertinence to the audience comes in second. We always try to find speakers who, while being excellent in their research, are able to explain their work to a broad audience. Finally, the easiest part is distribution: We make all presentations available online, and a remarkable number of journalists report from the conference, whose professional qualification is to convey complicated subjects to their readers and viewers. With the live-stream the audience was multiplied by ten in the last year, by hundred due to the online availability of the videos. We'll see what happens this year." In addition to watching the presentations online, viewers (approx. 8,000 this year) can contribute questions or comments. Thus, even though it may not be easy to get inside, the organizers make an effort to get people involved. In theory, one might say, their intentions are in tune with this year's opening lecturer Robert Darnton (Harvard University), who is a proponent of open access and the democratization of knowledge, and sees the advent of modern media technology and digitization as a means to democratize culture. It remains an (open) question and would be interesting to see who actually watches from outside the walls of the venue and with what subjective benefit.

## The Talks

The presentations at this year's conference, which covered a wide array of disciplines and topics, were directed at three main problem fields: the fundamental questions of humanity (What was there before the universe?), current global challenges (How do we end famines and protect individual security?), and the measures available to science itself for tackling these 'walls'. One of the recurring themes was the plea for interdisciplinary cooperation and joint effort of scientific and other societal players in facing global challenges. As this mantra of the contemporary academic and political landscape is easier (and more often) preached than actually put into practice, it was pleasant to see speakers introducing innovative tools capable of fostering crossdisciplinary approaches. Seemingly disparate areas of research were tackled by taking on one single obstacle. One such issue is the effective handling of vast and ever growing amounts of digital data, on which more and more disciplines from astronomy to art history depend. As computers shrink, explains computer scientist Anastasia Ailamaki, growing data deluge puts critical limitations to the speed of scientific research, which grows more relevant with the urgency to find solutions quickly. With her work, Ailamaki enables discoveries in diverse scientific fields by equipping research teams with database solutions and developing new algorithms capable of performing multiple operations simultaneously.

Other talks suggested that there could indeed be potential synergy effects with Ailamaki's work. DNA sequencing is one of the procedures that could profit from innovations in data processing. Its cheap and quick realization is fundamental to the future development of personalized medicine, the subject of Nobel laureate in chemistry Aaron Ciechanover. Yet another possible application is British evolutionary biologist Nick Barton's research, which deals with one of the most fundamental questions of humanity: Why do we bother reproducing sexually? According to Barton, even being fairly expensive (mating can cost enormous amounts of energy and carries a lot of risks), from an evolutionary standpoint, sex is just a smart thing to do: the combination of genes from different organisms is superior to asexual reproduction as it heightens the chances of adaption and survival. Until now, Barton has only been able to show this mathematically, but sets out to empirically test his hypothesis using genome data.

A characteristic difference between Falling Walls and a 'typical' scientific conference became apparent, when more current topics like environmental issues and ecological sustainability or the protection of health, individual wellbeing or human security were touched: In addition to offering facts about and insight into scientific phenomena, the talks presented at Falling Walls were almost always equipped with a vision, they often tried to convey an emotional message to the audience. Robert Schlögl, director at the Fritz Haber Institute emphatically stressed the importance of thinking about alternatives to fossil fuels and corresponding ways of storing energy. He looks for ways to develop storage methods for sustainably generated energy. New methods in oxidation catalysis could contribute to the development of 'solar fuel' modeled after nature's own energy storage: sugar. To put things in perspective, Schlögl made clear that we are far away from changing our current dependence on fossil fuels quickly. He stated that to develop eco-friendly energy storage, "nature had four billion years – we have to do it in 20". With the global financial crisis still smoldering, economy was critically discussed and further appeals were made. Stewart Wallis, director of the new economics foundation, diagnosed that a fundamental problem with economy today is its ignorance towards ecological limits of life and what he called the four problems or 'u-s" of economy today: It is unsustainable, unfair, unstable, and makes people unhappy. Economy's narrow focus on economic growth, Wallis continued, is echoed by the economic science, which measures economic growth solely by indicators like the gross domestic product. Wallis called for a more holistic understanding of economic progress and the consideration of indicators such as individual wellbeing, societal justice, and ecological sustainability. With calls for change from such diverse directions, the findings of decision scientist Elke Weber received particular attention and response. Weber explained why changing behavior is so difficult. Human aversion to change is really more a status quo bias, Weber explained: In mental decision processes, the status quo is preferred because of the way the brain organizes evidence - we stick to our habits. The solution lies in what she calls "decision architecture": Understanding how decision processes work psychologically and applying this knowledge to influence decision behavior. To illustrate her point Weber referred to the much higher number of organ donors in countries that had an 'opt-out' policy, i. e. in which citizens are potential organ donors until they actively declare otherwise.

## The Challenge

Falling Walls gives a lot to think about in little time. This formula makes the conference itself an experiment, testing how much an individual can learn in one day, as Turner put it in his 2010 opening address. Naturally, the majority of the topics discussed at the conference comprise complex subject matters. Given the diversity of the audience and the limited time-frame (each speaker is given exactly fifteen minutes and the measures taken to keep the tight schedule are infamous) speakers have to come up with ways to catch and bind the audience's attention and convey their sometimes complex ideas and their conceptual preconditions. The resulting entertainment factor of the presentations (the audience was, among other things, engaged in live onlinegambling on stage) is a refreshing contrast to other formats, but also an expression of the concept's limitations: Falling Walls cannot be (and does not claim to be) about exhaustive

answers and purely fact-oriented scientific rigor.

That time is indeed a challenge for both speakers and organizers became particularly apparent when the format was put on fast forward in the Falling Walls LAB, a new spinoff in cooperation with business consultant A.T. Kearney, which offers 100 young academics and professionals the unique opportunity to present their breakthroughs to a distinguished jury, combined with a scholarship that allows free participation in the main conference the following day. The challenge: the even tighter three-minute time frame. Chair of the jury and former president of the Leibniz Society Ernst Th. Rietschel underlined how important it was for scientists to be able to convey their message to partners, and praised the Falling Walls LAB as a new format to promote condensed information in talks. The presentations were as diverse as those on the conference the following day, often echoing topics such as personalized medicine or data management, which showed how both young and established researchers unite in tackling these issues.

In this marathon of ideas, projects have to be boiled down to the very essence. Participants took different approaches to this challenge. Some excelled at this challenging task, like winner of the Audience Award and the jury's first price Shuo Zhang from the Max Planck Institute for Biophysical Chemistry in Göttingen with his presentation on magnetic resonance imaging or Eileen Diskin from Trinity College, Dublin, who explained her breakthrough in antibiotic resistance using a bright pink flamingo mascot. Other ideas suffered from too much compression and got lost. Even with successful presentations, the mere number and frequency of talks, caused the audience (those we spoke to, at least) to mentally flick through the presentations and wait for an idea that 'resonates'. This mostly seemed to happen with ideas or research paradigms with which the listener was already familiar. As one of the concerns of the event is to overcome narrow minded 'silo thinking', this effect seems rather unfortunate. There can never be enough time for discussion and reflection, Turner says. Due to its brevity and density, Falling Walls is mainly a site for meeting people, inspiration, and irritation, where individuals can find new impulses and cooperating partners for their work back home - which as a matter of fact happens frequently. At the conference,

the organizers strive to make the best of the time budget, for example by offering a "Meet the Speakers" area where participants can discuss directly with the speakers. The organizers constantly look for ways to improve the conference. Following the careers of ideas presented at the conference is one of the future aims, along with finding ways to reach students and undergraduates, further enhancing the international layout of the conference, and above all improving the value of the conference for the participants. At the end of each conference, Turner says, the organizers have an endless list of newly discovered obstacles they set out to overcome the following year.

### The Experience

To sum it up, Falling Walls is not a typical scientific conference in many ways. And for those not expecting to be informed exhaustively about scientific issues, but to spend an animating day with others engaged in research it is a convincing concept. What appears to be its unique quality in any case is that the participation in the Falling Walls conference is not only intellectually stimulating, but also an overwhelming experience. As Sebastian Turner put it: "The contact to top researchers as well as the engagement with their work is very rewarding. It is a little surprise, that there is no enjoyment-tax for this. These are inspiring minds, that think 'out of the box' with delight". Inspiration on more than an intellectual level is also what participants seem to appreciate. Whenever we spoke to someone about their experience of the conference, everybody from speakers to audience members pointed out the 'spirit' at the conference as particularly rewarding. And indeed, putting aside the quality of the program and the actual content of the conference, it seems to be its ability to ignite people's passion for and belief in scientific research and its ability to make a change, that makes Falling Walls a success and has led its way from experiment to tradition.

– Tobias Boll

Full coverage of past and this year's presentations is available at www.falling-walls.com

# **Can Chemistry Be Green?**

While pursuing my Ph.D. project in chemistry, I am often confronted with a disturbing experience: Fellow scientists freely admit that the terminology they use when communicating their research to the public is dominated by catchy slogans. Moreover, these slogans often have not much in common with the research they pretend to explain. Even as a doctoral student, I sometimes find myself in the situation to present my work in terms of a distorting language. On the occasion of this year's Falling Walls Conference, I want to share some thoughts on this style of speech that scientists often employ when addressing a broader audience. As the conference included a lecture by Paul Chirik entitled "Breaking the Wall of Sustainable Chemistry. How Modern Alchemy Can Lead to Inexpensive and Clean Technology", I will use the example of "sustainable" or "green" chemistry. These expressions have grown increasingly popular in public, but most scientists will agree with me when I claim that they confuse the public and do not enlighten it. In fact, expressions like "sustainable physics" or "sustainable biology" are by far not as popular as "sustainable chemistry", which already hints at the latter being a conspicuous neologism.

Let us now look at the challenge of sustainability and chemistry's role in this context. With global population having surpassed seven billion people in 2011, the importance of economic, environmental, and social sustainability at all levels of human society becomes more and more obvious. As chemical industry holds a central position in the world's economy, one question naturally arises: How can industrial processes be designed in a sustainable manner? Important aspects related to this overriding question include the reduction of fossil fuel consumption, the minimization of waste production, and the replacement of toxic or expensive substances by harmless or inexpensive ones. The latter aspect was exemplified in Paul Chirik's lecture, which dealt with attempts to replace the precious metal platinum by the cheap and abundant metal iron in chemical reactions. Without commenting on his lecture in detail here, my personal perception-and probably the interested layman's perception, too-was that Paul Chirik convincingly showed how the replacement of platinum by iron is carried out in detail, i.e., what principles are applied, which hurdles have been overcome, and which problems remain to be solved.

The attentive reader may have noticed that I have not used the terms "sustainable chemistry" and "green chemistry" when presenting Paul Chirik's lecture. Why? The reason is simple: I think they are unnecessary and, furthermore, possibly misleading. Let us examine the term "sustainability" in more detail. It can be vaguely defined as the capacity to endure, but its usage has become so popular and widespread that a comprehensive definition is beyond the scope of this essay. However, in the present context it is important to realize that sustainability originally referred to human agency and ethical standards. Accordingly, the word was used in philosophy, economics, and politics, or more general, when dealing with humans or the human society as a whole. As a consequence, the concept is inapplicable to natural science, which seeks to shed light on the principles that govern the natural world.

Now let us try to understand what sustainable chemistry really is. For the reason outlined above, I would not consider it as a new chemical branch comparable to inorganic chemistry, biochemistry, or analytical chemistry. Instead I claim that sustainable chemistry is a specific point of view from which one may reexamine established chemical methodology. This reexamination applies ecological and economic criteria to chemistry, thus a catchy definition of sustainable chemistry could be: Sustainable chemistry is the mere continuation of economics and ecology by chemical means. To underpin this claim, let us look at the twelve principles of green chemistry formulated by Anastas and Warner.<sup>1</sup> They are:

- 1. It is better to prevent waste than to treat or clean up waste after it is formed.
- 2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
- 3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
- 4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.
- 5. The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
- 6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.
- 7. A raw material or feedstock should be renewable rather than depleting wherever technically and economically practicable.
- 8. Reduce derivatives unnecessary derivatization (blocking group, protection/ deprotection, temporary modification) should be avoided whenever possible.
- 9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
- 10. Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.

<sup>&</sup>lt;sup>1</sup> P. T. Anastas and J. C. Warner, "Green Chemistry: Theory and Practice", Oxford University Press, New York, **1998**, p.30.

- 11. Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
- 12. Substances and the form of a substance used in a chemical process should be chosen to minimize potential for chemical accidents, including releases, explosions, and fires.

These principles do not constitute a set of strict rules but rather a guideline. While their detailed discussion would require another essay, I only point out here that they can all be considered applications of basic economic and ecological principles to chemistry. In my opinion, this claim holds true for all further ideas related to sustainable chemistry.

What should one think of this development? As far as I can see, some chemists fear that the original scientific principles of chemistry are in danger of being replaced by economic principles, yet I think this fear is absolutely groundless. The principles of economy and ecology will not replace established chemical methodology but they can and should drive chemical research. Since sustainable development is so crucial for our society, every scientist should have a basic knowledge of economy and ecology. On the other hand, the traditional chemical methodology is not outdated, but provides the means necessary to achieve an ecological goal. As Paul Chirik illustrated in his talk, the assessment of a certain industrial process with respect to sustainability is most often not straightforward, but rather complicated and requires an integrated approach taking into account countless details. The understanding of these details is the scientist's intrinsic domain, yet to assess the impact of his research, the importance of a broader background comprising economic and ecological knowledge grows. Accordingly, my first conclusion is that sustainable chemistry is important and should be taught to chemistry students. However, it should not be presented as a chemical discipline, but as a valuable extension.

My second claim is that the public popularity of the term "sustainable chemistry" results from a worrisome confusion between chemistry and chemical industry. This confusion becomes entirely obvious when looking at the even more popular expression "green chemistry". I am convinced that this expression derives its popularity from a seemingly inherent tension as many people equate green with clean and chemistry with dirty. Yet in reality, chemistry is neither dirty nor clean, but chemical industry can be dirty and we need to make it cleaner. In this context, expressions like "green chemistry" or "sustainable chemistry" are in danger of being abused as public relations label and of being perceived by the layman as chemical methodology. In this way, the confusion between chemistry and chemical industry is not resolved but even increased. To dispel public concerns about chemistry-or in Falling Walls language, to break the wall between chemistry and the public-one should make very clear that sustainability is an ethical concept-which chemists in industry and science ought to be aware of and which may drive chemical research-but not a scientific principle.

After all, there is a simple conclusion: It is every scientist's duty to employ an understandable but still precise language. If we feel seduced to replace a lengthy and cumbersome explanation by a catchy one-liner, we should recall that this is easy in the short run, but feeds the public mistrust against science in the long run.

- Thomas Jagau

# Communicating Math to the Masses - an Encounter with Cédric Villani



Charly Chaplin<sup>1</sup> once said to Albert Einstein: "I am applauded, because everybody understands me; you are applauded, because nobody understands you." Cédric Villani, professor for mathematics at Université Claude Bernard in Lyon, would probably strongly disagree with Chaplin's dictum. A celebrated mathematician, his awards include the Fields Medal in 2010 and the prize of the European Mathematical Society in 2008, but he believes that his difficult work on partial differential equations is communicable to everyone. His talk "Breaking the Walls between Economics, Physics and Geometry. How Optimal Allocation of Resources and Entropy Meet in the Non-Euclidean World", which he presented at this year's Falling Walls Conference in Berlin, was a tour de force of scientific communication. The Journal of Unsolved Questions had the honor to talk to Cédric Villani about communication of mathematics to the public, scepticism towards numbers, population growth, and the climate change.

<sup>1</sup>The picture was derived from a license free clipart published on clipart.net

**JUnQ**: Prof. Villani, when you open the newspaper in the morning, how much do you think about the complex problems presented there in terms of mathematical concepts?

**Villani**: How much I think about the news in mathematical terms depends very much on the mood, on the problem, on many things. You can compare it to music: If you know music, you can either listen in a naive way or listen with the ears of a specialist, recognizing the chords, harmonies and so on. If you really want to understand what is happening in the world, though, you will need mathematics directly or indirectly.

**JUnQ**: A recent example for math appearing in the news is the world population reaching 7 billion. It is not trivial to calculate when exactly this mark was reached, nevertheless not many formulas were presented in the papers, only this huge number: 7 billion! Why is it so much harder to communicate an abstract formula than a single number?

Villani: Do you understand 7 billions? Can your brain comprehend such a number? I think mine can't. This is just enormous, impossible to get. The abstract formulas are not intrinsically hard to communicate. I have recently seen a clown show where kids were given an idea of the predatorprey system. This is abstract and can be conveyed without any precise figures. Formulas are just set in a different language. If you learn the language, it will sound easy. And it will be extremely useful. Even simple formulas for population growth contain so much more information than just a single number. I can represent the development of the population, see the movement, program it on my computer and so on.

**JUnQ**: But recently we have seen a lot of examples where these formulas failed. Take the financial crisis as an example. The mathematical models of the rating agencies were unreliable. The risk calculations of the banks obviously failed. There is skepticism and even anger directed towards the experts in charge of the calculations. Is this skepticism justified?

**Villani**: About the financial crisis, yes, things are so complicated. The blame should not be put on mathematics, though, but rather on the fact that the mathematical models are applied way out of their range of validity in the hope that they will still work. We should be damn skeptic towards numbers calculated in that way, especially when they are presented as facts. Often numbers are used to pretend something is sure! Think of the following sentence, which I also learned from a clown: "90% of people believe in a sentence which has percentages in it." To come back to the prior question, knowing a bit about the underlying models and calculations is an excellent way to appreciate the uncertainty, which is behind them, and to judge when the model is trustable and when it is not. Even if this requires quite an expertise.

**JUnQ**: Appreciating the uncertainty is not always easy. The predictions for the global warming until 2100 range from 1.1 °C to 6.4 °C, a huge difference. If you ask five differenct scientists you can expect ten different answers. How can we decide which expert to believe in?

**Villani**: I have more trust in experts which present different results than in experts who present one single result. Giving several results means you have tested several hypotheses, you have been critical, you are humble enough to admit your partial ignorance. Science in general is often criticized for its uncertainty, the climate issue is a good example. Even though they do not always look secure, scientific approaches are still the most reliable, more reliable than other approaches like faith, politics, or feeling at least. Sometimes the precision of results is amazing. The difficulties of scientists just reflect the fact that the world is so complicated.

**JUnQ**: Do you feel that there is a wall of alienation between the mathematician and the public? Do you have an idea how we could make the public more enthusiastic about math?

**Villani**: My experience is that the public is always enthusiastic to learn about science. And scientists are among the last heroes in our era, the most trusted experts. The percentages of confidence for scientists are substantially better than for politicians. In all my public lectures, I get the impression that the public has an enormous appetite for science. To make the public enthusiastic, it is sufficient to present science in a pedagogical way, in an incarnated way, with people, adventures, stories, and history.

– Leonie Anna Mück

# **Questions of the Week**

The Journal of Unsolved Questions presents a "Question of the Week" on its homepage every week. Set up and formulated by the members of the editorial board, the main purpose of the "Question of the Week" consists in intriguing the reader by presenting topics of ongoing research. "Questions of the Week" published so far cover a wide variety of scientific fields, but share the feature to be of certain importance to several disciplines.

#### In the following, we present selected "Questions of the Week" from the last six months.

#### What is the mechanism of sonoluminescence?

by Libor Veis



A single cavitating bubble<sup>1</sup>

One of the big unsolved questions in current physics is the mechanism of sonoluminescence. Sonoluminescence

is a phenomenon that occurs when a small gas bubble is acoustically suspended and periodically driven in a liquid solution at ultrasonic frequencies, resulting in bubble collapse, cavitation, and light emission. For example in a single-bubble sonoluminescence (SBSL), a single bubble is trapped in an acoustic standing wave and emits bursts of light with each compression of the bubble. It was realized that temperatures inside bubbles can reach unbelievable values of thousands of Kelvins. The mechanism of sonoluminescence however remains unsettled. There are plenty of theories including hotspot, bremsstrahlung radiation, collision-induced radiation, corona discharges, nonclassical light, proton tunneling, etc. An exotic mechanism first proposed by Julian Schwinger (Nobel Prize for Quantum Electrodynamics, 1965) and later on considered by others suggests a quantum vacuum radiation as an explanation.

# Why do some languages not distinguish between blue and green?

by Thomas Jagau

The English language together with most indo-european languages makes a clear distinction between blue and green while many languages in tropical regions use the same expression for these two colors. In addition, some languages do not distinguish between blue and black either. Possible explanations include the hypothesis that stronger UV radiation in tropical regions causes retinal damage thereby weakening people's capacity of perceiving colors in the short-wave range of the spectrum. Another hypothesis considered more plausible today does not involve physical explanations but goes back to the development of languages in general. As first proposed by Lazarus Geiger and later re-discovered by Brent Berlin and Paul Kay, the color terms in a specific language always emerge in the same sequence: First, a language distinguishes only between black and white, the next color to be named is red. I. e. if a language has three distinct terms for colors, it is always black, white, and red. As a fourth color term yellow or green emerges, but blue is always the last color term to be introduced. Languages that do not distinguish between blue and green thus may just have not developed a separate name for blue.

#### Read more:

The world atlas of language structures online, http://wals.info/chapter/134

Brent Berlin and Paul Kay, "Basic Color Terms: Their Universality and Evolution", **1969**, University of California Press, Berkely, USA

<sup>&</sup>lt;sup>1</sup>The image was uploaded by user theearthling to http://en.wikipedia.org/wiki/Sonoluminescence

#### Why does a coffee drop leave behind a ring-shaped stain?

by Leonie Anna Mück

Coffee is a particle suspension or, more specifically, a colloidal dispersion. When the water in a coffee drop evaporates, a ring-shaped stain remains. The mechanism of the stain's formation has been well described by Deegan et al. [1]: The evaporation takes place from the edge of the stain. Because the amount of liquid at the edge gets restored with liquid from the centre of the drop a capillary flow is generated as the liquid evaporates. This flow drags more and more particles to the edge of the stain leaving the ring-shape behind. This deposition mechanism can be applied to make colloidal crystals, but for a long time the mechanism of the order-disorder transition has been unclear, i. e. it has been unknown under which conditions the evaporation would result in colloidal crystals rather than amorphous structures. Detlef Lohse and co-workers have recently brought light to the basic mechanism of this order-disorder transition [2]: At the beginning of the evaporation the liquid flow to the edges is low and the particles dragged to the edge have time to crystallize. At the end the speed of the flow is a lot higher and particles with high-speed are packed into a disordered phase.

References:

[1] R. Deegan, O. Bakajin, T. F. Dupont, G. Huber, S. R. Nagel, and T. A. Witten. *Phys. Rev. E* **2000**, *62*, 756–765

[2] Á. G. Marín, H. Gelderblom, D. Lohse, and J. H. Snoeijer. *Phys. Rev. Lett.* **2011** *107*, 085502

#### What is the best strategy to combat pest nematodes?

by Thomas Jagau



Heterodera glycines, a parasite of the soy bean<sup>2</sup>

With approximately 1.000.000 species, nematodes are the most numerous multicellular animals on earth. Attacking plants and spreading viruses, pest nematodes pose a huge problem for agricultural research. With global crop yield loss surpassing \$100 billion per year, the need to combat pest nematodes is clear, but excessive pesticide use causes harm to people and the environment. Instead, sustainable approaches must be pursued to increase the food harvest and ensure food security.

Read more: http://falling-walls.com/lectures/waceke-wanjohi/

http://www.africannematology.info/

## Faster-than-light neutrinos?

by Rute André

In the last couple of weeks a lot of attention has been put on the data presented by the group of scientists working in the Gran Sasso laboratory, where they claim to measure neutrinos with a speed faster than light. Proven true, of course this could bring very interesting new insights to the physics world. According to the data shown, the authors expect the measured neutrinos to have a speed about 60 nanoseconds higher than the established speed of light. Some criticism arose from the possible mistakes being introduced from the statistical approaches used to calculate the values. A very interesting analysis on how the models used to treat the data might introduce some error, can be found here: http://scienceblogs.com/startswithabang/2011/09/ are\_we\_fooling\_ourselves\_with.php You can also read the original paper on arxiv: http://arxiv.org/abs/1109.4897

<sup>&</sup>lt;sup>2</sup>The image was produced by the Agricultural Research Service, the research agency of the United States Department of Agriculture.

#### Moore's law and fast evolving next-generation sequencing technologies

by Johannes Werner

Moore's law describes that the period for doubling the performance of a computer chip amounts to approximately 2 years [1]. It is interesting that decreasing costs in DNA sequencing caused by next-generation sequencing (NGS) outranges the effect of Moore's law by orders of magnitude [2]. As a consequence, the price of sequencing by NGS technologies decreases much faster than those for e.g. data storage. Due to the recent developments in NGS technology, it is expected that the exponential growth of sequenced data in public databases [3] will continue during the next years. The question is, how can the increasing gap between Moore's law and advances in NGS be resolved regarding to storage or analyzing of the genomic data? References:

[1] G. E. Moore, "Cramming more components onto integrated circuits", *Electronics* **1965**, 38(8).

[2] A. Sboner, X. J. Mu, D. Greenbaum, R. K. Auerbach, M. K. Gerstein, "The real cost of sequencing: Higher than you think", *Genome Biology* **2011**, 12(125).

[3] M.-E. Guazzaroni, A. Beloqui, P. N. Golyshin, M. Ferrer, "Metagenomics as a new technological tool to get scientific knowledge", *World Journal of Microbiology and Biotechnology* **2009**, 25(6).

#### **Do lipid-rafts exist?**

by Rute André

It is generically known that the cell membrane is composed of a lipid bilayer with embedded proteins, but agreement on its organization has not been achieved yet. Lipid-rafts have been suggested to be regions in the membrane with distinct compositions that would act as platforms for proteins involved in intercellular signaling pathways. Despite a big number of publications in this subject, doubt still exists regarding the existence and nature of these lipid-rafts, mostly because of the limitations of the methods used for isolating this domains, in which small changes in procedure from work to work lead to different material isolated and consequently different material characterized. Until techniques are developed that allow the marking and visualization of these assumed domains in living cells, the question will remain open. Science Magazine is running a special about the Mysteries of the Cell, in which the lipid rafts between other intriguing unsolved questions about the cell are also discussed.

Read more:

http://www.sciencemag.org/site/special/cellbio2011/

#### What is the significance of electron correlation in explaining why fireflies produce light? by Leonie Mück



Fireflies in the wood, exposure time 30 seconds<sup>3</sup>

On a warm summer evening spent outside in the company of good friends and crickets one thing can't be missing to get just the right atmosphere: The fireflies. Their bioluminescence magically creates a starry sky just above the surface of the earth. To be more exact, the enzyme luciferase activates the substance luciferin. The light emitting section of luciferin is dioxetane and it converts chemical energy into cold light by reaching its excited states through a conical intersection. Loren Greenman and David Mazziotti recently studied dioxetanone by means of quantum chemical methods. They argue that the anti-Hermitian contracted Schrodinger equation describes both, dynamic and static electron correlation, and that this method is sufficiently accurate to describe the region close to the conical intersection of dioxetanone where strong correlation has to be taken into account.

So, next time you spend one of these splendid summer evenings outside, let yourself be enlightened by the fireflies to find the ultimate solution to the electron correlation problem in quantum chemistry.

#### References:

[1] L. Greenman, D. Mazziotti, J. Chem. Phys 2011, 134, 174110.

[2]L. Greenman, D. Mazziotti, J. Chem. Phys 2010, 133, 164110.

<sup>&</sup>lt;sup>3</sup>The image was taken from http://en.wikipedia.org/wiki/File:GluehwuermchenImWald.jpg and uploaded by user Quit007

# Articles

# Dependence of *E. coli* Chemotaxis on CheB Phosphorylation *in Silico* and *in Vivo*

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Received 17.10.2011, accepted 30.11.2011, published 03.12.2011

The protein CheB is an integral component of sensory adaptation in the chemotaxis system of *Escherichia coli*. It catalyzes demethylation of the chemoreceptors thereby opposing the effect of ligands on kinase activity. The kinase enhances the activity of the methylesterase via phosphotransfer, thus creating a negative feedback. Although CheB phosphorylation depends on the receptor state, it is not essential for precise adaptation. Therefore, the feedback mechanism is proposed through modeling to compensate for protein fluctuations in the chemotaxis network.

Swarm plate assays revealed that chemotaxis performance in general was even more robust against deviations of single protein concentrations than predicted. However, phosphorylation deficient mutants of CheB still enabled an appropriate chemotaxis response as compared to wild type CheB. Furthermore, when simulations were recoded to include CheB phosphorylation, there was no effect on swarming.

Hence both, measured and calculated swarm efficiencies indicate that CheB phosphorylation does not improve robustness of chemotaxis against perturbations in protein levels.

## 1 Introduction

Thanks to pioneering work [1], bacterial chemotaxis became one of the most studied and well-documented systems in molecular biology. The machinery for cell motility senses and reacts upon temporal changes in chemoeffector concentrations. Signal transduction components are encoded by six essential genes: *cheA*, *cheB*, *cheR*, *cheW*, *cheY* and *cheZ*. Partially redundant [2] membrane-spanning receptors (e.g. Tar) transmit input cues from the periplasmic space to the cytoplasm (Fig. 1).

CheW acts as an adapter between receptors and the histidine kinase CheA, which catalyzes the transfer of phosphoryl groups from ATP to own histidine residues. The phosphoryl group is rapidly transferred to response regulators; in this case mainly CheY. The phosphorylated CheY (CheY-P) is released from the complex, diffuses and binds to flagellar motor switches. The rotary motors are embedded in the cell envelope [3]. As an output, CheY-P switches the flagellar rotation from counter-clockwise (CCW) to clockwise (CW) [4], resulting in different swimming behavior of the cell. The phosphatase CheZ mediates turnover of chemotaxis response by dephosphorylation of CheY-P close to the receptors [5]. Evolved interactions between those molecular components ensure an optimal chemotaxis performance [6]. Attractant binding to the receptor inhibits kinase activity. Subsequently low CheY-P levels result in persistent counter-clockwise flagellar rotation and hence unchanged swimming direction of the cell. Repellents (or declining attractant concentrations) sensed by the receptor cause ac-

<sup>&</sup>lt;sup>1</sup>The theoretical work was done at BioQuant Insitute, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany and experiments were conducted at Zentrum für Molekulare Biologie der Universität Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany. Corresponding email address: l.adlung@dkfz-heidelberg.de

tivation of CheA and induce phosphorylation of response regulator CheY. The flagellar rotation is shifted to clockwise and the cell starts "tumbling".



Figure 1: Schematic overview of the chemotaxis pathway. Components are abbreviated. For further details, refer to the text.

Driven by the molecular machinery, the microbe runs in favorable directions towards high concentrations of attractant and away from repellents. An amazing feature of the chemotaxis system is adaptation. This is the property to maintain sensitivity over a wide range of chemical stimuli. Even at saturating concentrations of attractants or repellents, the system returns gradually to pre-stimulus values, which means that kinase activity, CheY-P level and motor bias adapt precisely to a certain steady state. Additionally, this ability seems to be robust against changes in concentrations of chemotaxis proteins. *E. coli* chemotaxis turned out to be a simple but well-adjusted system with robustness of adaptation precision through its network architecture [7].

The molecular components which are responsible for adaptation are CheR and CheB. Methyltransferase CheR constitutively methylates specific receptors, i.e. methyl accepting chemotaxis proteins (MCP). Methylation increases receptor potential to stimulate kinase activity and decreases receptor affinity to attractants [8]. The reverse reaction is catalyzed by the methylesterase CheB. Concerning the structure, CheB consists of two domains connected by a short linker sequence. The regulatory N-terminus is homologous to CheY. Both proteins bind competitively to the P2 domain of CheA [9] and can be phosphorylated by the activated kinase as response regulators. Phosphorylation at Asp56 leads to conformational changes of CheB [10] and increases its affinity to the receptor cluster [11]. However, N-terminal deletion mutants, which cannot be phosphorylated, still maintain chemotaxis ability and appropriate swimming behavior [12]. The unphosphorylated N-terminal domain exerts an inhibition on the effector domain by partially occluding the active site of CheB. Kinetic analyses indicate nearly 100-fold increase of CheB methylesterase activity through relief of inhibition and stimulation of catalysis by phosphorylation [13].

Receptors in the active state lead to autophosphorylation of CheA and phosphotransfer to CheB. Hence activated CheB catalyzes MCP demethylation and receptor activity diminishes. Interestingly, such a negative feedback loop is not essential for precise adaptation [14]. This raises the question of the exact role of CheB phosphorylation in the chemotaxis pathway. How does swarming performance of *E. coli* depend on the feedback via CheB? There are at least theoretical indications that CheB phosphorylation provides robustness, in terms of unaffected swarming performance, against varying chemotaxis protein concentrations [15]. Here, experiments and simulations were conducted to analyze the proposed function of the feedback loop. Swarm plate assays [16] were combined with the use of *RapidCell* [17], a multi-scale modeling software for swarming bacteria. Results suggest a new perspective on the methylesterase and its regulatory function.

## 2 Experiments and Simulations

The role of CheB phosphorylation was investigated in vivo via swarm plate assays. Ideal swarming conditions were determined including suitable induction levels for chemotaxis gene expression from plasmid. The knock-out strain  $(\Delta cheB)$  was transformed with a plasmid encoding either wild type CheB or one of the mutants CheB<sup>D56E</sup> and CheBc. Those restore function to some degree. After titration, strains were co-transformed with another plasmid containing one of the other chemotaxis genes. The resulting swarming performances were evaluated referring to up-regulated protein levels of each CheA, CheR, CheW, CheY, CheZ and Tar. It was the aim to see whether wild type CheB phosphorylation provides additional robustness against over-expression of chemotaxis genes as compared to the deficient mutants. To study chemotaxis in silico, an equation representing CheB phosphorylation was integrated into the RapidCell program code. The software sources for input and output were refined. Simulated results were then compared to experimental observations.

#### 2.1 CheB Titration

Wild type CheB or one of the two mutants, CheB<sup>D56E</sup> and CheBc, were expressed from a plasmid in the  $\Delta cheB$  strain. They were tested for complementation to chromosomeencoded CheB in wild type strains. The mutant CheB<sup>D56E</sup> cannot be phosphorylated due to the point mutation and shows only a basal level of activity. The mutant CheBc lacks the N-terminal regulatory domain and the remaining catalytic domain with a short linker cannot interact suitably with the chemotaxis cluster. Corresponding genes were expressed from plasmids with an arabinose-inducible promoter.

*E. coli* requires a functional chemotaxis system to swarm on soft agar plates. The cells spread radially, because they form attractant gradients by metabolizing nutrients in the agar. Complementing CheB, CheB<sup>D56E</sup> and CheBc in the  $\Delta cheB$  strain affected swarming. Optimal swarming for CheB was obtained with 0.001 % arabinose. Swarm ring sizes changed with arabinose induction level and were compared to the wild type strain for calculation of relative chemotaxis efficiency. Protein amounts of all CheB variants were quantified with Western blots relative to CheB levels of the wild type strain.

The phosphorylation mutant was expressed at least two fold of wild type level to enable swarming (Fig. 2). Presumably, basal activity of CheB<sup>D56E</sup> was insufficient for functionality of chemotaxis at wild type expression level. In contrast, strains expressing CheBc swarmed already if the mutant level averaged 20 % of wild type CheB (Fig. 2). Reported ten-fold increase in methylesterase activity compared to unphosphorylated CheB [13] would be reasonable in this case. CheBc represents the uninhibited state of CheB through relief of the regulatory domain. The methylesterase without N-terminus is constitutively active and suspected to localize to polar receptors by direct interactions with the MCPs [18]. Huge amounts of CheBc would then result in permanent demethylation of receptors what directly promoted stimulation by attractants and improved chemotaxis performance. That is, why CheBc expressing strains swarmed better at high induction levels than those expressing wild type CheB under laboratory conditions. It remained unclear if swarm efficiency would diminish for higher CheBc levels or if swarming would reach a steady state through saturation of net demethylation.



Figure 2: Dependence of swarm efficiency on expression levels of CheB variants. Blue line with diamonds:  $\Delta cheB$  + CheB, red line with squares:  $\Delta cheB$ + CheB<sup>D56E</sup>, green line with triangles:  $\Delta cheB$  + CheBc. Standard deviations are given by error bars, n=3.

The curve for swarm efficiency with increasing protein levels of CheB was less steep as compared to the mutants (Fig. 2). The smooth gradient of the graph hinted at a more balanced mediation of swarming via CheB and some feature of robustness contrary to high sensitivity of CheB<sup>D56E</sup> and CheBc.

#### 2.2 Chemotaxis Performance of CheB Variants

The next aim was to see if CheB phosphorylation provides robustness of the chemotaxis system against single protein fluctuations. Robustness means stable output (i.e. constant swarming behavior) despite perturbations (i.e. various expression levels). Therefore, plasmid-encoded chemotaxis proteins were simultaneously expressed with CheB, CheB<sup>D56E</sup>, or CheBc from respective plasmids in the *cheB* knock-out strain. CheB variants were always induced with the preliminarily obtained optimal concentration of 0.001 % arabinose (see 2.1). The expression of the other chemotaxis genes was always induced with increasing levels of IPTG.



Figure 3: Chemotaxis performance for over-expression of CheZ-YFP (left panel) and CheA (right panel). Blue line with diamonds:  $\Delta cheB$  + CheB, red line with squares:  $\Delta cheB$  + CheB<sup>D56E</sup>, green line with triangles:  $\Delta cheB$  + CheBc. Standard deviations are given by error bars, n=6 (CheZ-YFP) or n=7 (CheA). Quantifications of CheZ-YFP via flow cytometry normalized to lowest induction level. Quantifications of CheA via Western blots referring to wild type.

Chemotaxis in general was unexpectedly robust against upregulation of the referring proteins, here fused to the yellow fluorescent protein (YFP). Only high over-expression of e.g. CheZ-YFP led to reduction of swarming ability (Fig. 3). Wild type CheB and its phosphorylation was not observed to counter balance this effect. Reduction of swarming was similar for wild type CheB and the mutants. Advantages resulted exclusively from 0.001 % arabinose induction but not from phosphorylation of wild type CheB. Results for CheW looked quite similar (data not shown).

In case of CheA, the native protein was studied since YFP fusions to CheA exhibit no appropriate function [11]. Expression levels were not as high as for the other chemotaxis proteins. The CheBc mutant strain mediated better swarming than wild type CheB within a narrow range around two-fold wild type expression (Fig. 3). This observation was consistent with explanations from the literature: While CheB interacts via its N-terminus with the P2 domain of CheA [19], the methylesterase activity is inhibited. Due to the lack of the regulatory domain, CheBc is not hampered, and increased CheA levels do not have a direct influence on CheBc activity. Consequently, robustness of chemotaxis is provided by CheBc against particular up-regulation of CheA.



Figure 4: Swarming robustness against CheR overexpression in the  $\Delta cheRB$  strain (left panel) and the  $\Delta cheB$  strain (right panel). Blue line with diamonds:  $\Delta che(R)B$  + CheB, red line with squares:  $\Delta che(R)B$ + CheB<sup>D56E</sup>, green line with triangles:  $\Delta che(R)B$  + CheBc. Standard deviations are given by error bars, n=6 ( $\Delta cheRB$ ) or n=4 ( $\Delta cheB$ ). Quantifications via flow cytometry, normalized to lowest induction level. For the Tar receptor, expression levels were also very low (data not shown). A distinction between wild type CheB and CheBc was again hardly possible and CheB<sup>D56E</sup> performance was just slightly worse. This further supported the hypothesis that chemotaxis performance does not benefit from the phosphorylation of wild type CheB in terms of robustness.

Methyltransferase CheR was investigated as C-terminal YFP fusion in a  $\triangle cheRB$  strain and the  $\triangle cheB$  strain. Chemotaxis behavior was robust over a broad range of CheR-YFP expression in the double knock-out strain (Fig. 4). Strikingly, complemented wild type CheB showed advanced swarming in comparison to CheB mutants. During same experiments in the  $\Delta cheB$  background, the mutants tended to be more motile (Fig. 4). A considerable difference is the presence of native CheR in the  $\Delta cheB$  strain, albeit in low concentrations [20]. An interaction between CheR-YFP from plasmid and chromosome-encoded CheR was assumed to cause changes in swarming. A direct interaction between native CheR and the CheB mutants is unlikely [11]. Such an eminent disparity between swarming performances in  $\Delta cheB$  strain and double knock-out strain expressing CheB mutants was unprecedented and remains to be resolved.

Theoretical considerations could facilitate investigation of the chemotaxis system and enhance our understanding. An auspicious software for large-scale simulations of swarming bacteria is *RapidCell* [17]. So far, *RapidCell* was lacking an equation for the CheB phosphorylation. In this work, RapidCell was advanced for theoretical studies of the CheB feedback loop to enable comparison between mentioned experiments and those simulations.

#### 2.3 Software Refinement and Analysis

Receptor methylation and demethylation is described in *RapidCell* by an ordinary differential equation (ODE). The program operated with phosphorylated CheB (CheBP) as a static parameter assuming CheB activity is not changing over time. Such a lack of CheB regulation obviously led to worse swarming behavior as indicated *in vivo* by the phosphorylation mutant CheB<sup>D56E</sup>. With an equation for CheB phosphorylation, the negative feedback loop for regulation of CheB activity was introduced into the model. CheBP became a dynamic variable, which was updated for every time step in response to other network properties. The ODE was adopted [15]:

 $\partial_t \text{CheBP} = k_{\text{CheBP}} \cdot \text{CheAP} \cdot \left(\text{CheB}^{\text{T}} - \text{CheBP}\right) - \gamma_{\text{CheBP}} \cdot \text{CheBP}$ (1)

The change of CheBP concentration over time was dependent on the following two parameters:

- (I) k<sub>CheBP</sub>, the rate of phosphotransfer from autophosphorylated CheA (CheAP) to CheB. Phosphorylation was proportional to CheAP and the concentration of unphosphorylated CheB, i.e. total amount of CheB (CheB<sup>T</sup>) minus CheBP.
- (II)  $\gamma_{\text{CheBP}}$ , the dephosphorylation rate of CheBP. This

turnover was solely dependent on CheBP because it is autocatalytic.

The changing CheBP concentrations directly influenced methylation state, which in turn altered the free-energy offset in the model. This had an impact on the system activity CheAP that fed back to CheBP values. These regulations balanced the system's output. Moreover, the feedback loop brought the model in a closer agreement to experimental data (Fig. 5).



Figure 5: Comparison of two RapidCell versions with experimental data. The change of phosphorylated CheY (CheY-P) in a single cell is plotted with time. The RapidCell version of this work includes CheB phosphorylation. Both simulations with identical settings.

Response of phosphorylated CheY (CheY-P) was measured via Förster Resonance Energy Transfer (FRET) after adding and removing attractant. Active CheB is required to return CheY-P to steady state. This CheB-dependent adaptation was fitted to the experimental results. The parameters  $k_{CheBP}$  and CheB<sup>T</sup> were refined to ensure a physiological amount of active CheBP. With the introduced phosphorylation equation, CheBP levels changed with CheAP. Theoretically, robustness is expected [15] when:

$$\frac{\partial \text{CheBP}}{\partial \text{CheAP}} > 0 \tag{2}$$

is fulfilled. Similarly, robustness is expected at least against over-expression of CheY and CheZ. Equation 2 was true for the model that included CheBP regulation but not for the old version with only one static value for CheBP. These differences between the software versions did not become obvious for calculated swarming behavior (data not shown). With over-expression of CheY-YFP, swarm efficiencies for all CheB variants diminished likewise (Fig. 6). The better wild type performance resulted again exclusively from the preliminary obtained optimal induction level. There was no additional robustness. This observation was in line with the simulation results (Fig. 6). CheB variants could not be discriminated.



Figure 6: Swarm efficiencies for different CheY levels. Experiments with CheY-YFP in the  $\Delta cheB$  strain expressing again different CheB variants (left panel) and referring simulations (right panel). Blue curve: software including CheB phosphorylation. Green curve: program lacking the equation. Red curve: simulation with only 60% CheB activity relative to wild type. Black graph: a calculation for another attractant gradient. Dark blue rectangle as a guide to the eye for experimentally determined expression range.

For CheY levels, a sensitive readout with steep input-output characteristics is known [21]. Small variations trigger large change in motor bias. The more CheY is present in the cell, the more likely it will be phosphorylated in competition with CheB. Phosphorylated CheY promotes tumbling. Hence, swarm efficiency was expected to drop when CheY gets out of its tight working range. This effect was tremendous for a constant activity gradient that was recommended for simulations of chemotaxis efficiency [17]. For gaussian gradient, simulated chemotaxis fitted swarm plate assay results. The impact of the applied attractant gradient in the model should be taken into further consideration since it dramatically altered the sensitivity of the system.

## 3 Conclusions

In summary, the smooth shape of the curve for wild type CheB during titration (Fig. 2) hinted at some impact of CheB phosphorylation on mediating chemotaxis performance. Those results suggest a regulatory function connected to the feedback loop. The over-expression studies (Fig. 3, Fig. 4) indicated that the robustness of chemotaxis performance in attractant gradients is not originating from CheB phosphorylation though the system exhibited high robustness in general. Predicted sensitivity of chemotaxis against protein up-regulation in some attractant gradients was contradictory (Fig. 6) to experiments.

On the other hand, the negative feedback loop definitely finetuned the system's response kinetics after removal of attractant (Fig. 5). Declining attractant concentrations have the same effects on the system as increasing repellent gradients. Future approaches should therefore be addressed to repellent swarming where the impact of CheB phosphorylation could be more pronounced. Such experiments are more elaborate [22]. But to elucidate those mechanisms, it could be worthwhile shifting the focus to repellent taxis.

Quite often in biology, structure and function are intimately associated. However, many questions concerning the rela-

tion between architecture and function of biological graphs and their evolution remain elusive. Negative feedback loops represent a generic motif in molecular pathways. They define dynamic signaling properties. Dissecting the role of CheB phosphorylation in chemotaxis could add valuable information to our understanding of network building blocks in nature. Perspectively, one could then re-engineer the chemotaxis machinery, e.g. to enable deliberate drug delivery or to aid intervention of microbial spread.

## 4 Acknowledgements

The work could not have been done without the contributions of: Prof. Dr. Victor Sourjik, Dr. Sonja Schulmeister, Dr. Ilka Bischofs-Pfeifer, Dr. Nikita Vladimirov, Prof. Dr. Ursula Kummer, Dr. Sven Sahle, and all members of the "Sourjik Lab".

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### On the Oxidation of Allylmelamines

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Received 08.11.2011, accepted 03.12.2011, published 27.12.2011

2,4,6-triamino-1,3,5-triazine (melamine) was first prepared in 1834 by Liebig. It has already been used for several decades for the production of melamine-formaldehyde resins and has therefore gained industrial importance. Particularly, during the last years new possibilities for the cross-linking of melamine have been developed to replace harmful formaldehyde. The synthesis of epoxy modified melamine derivatives is one possibility for this purpose. 2-Diallylamino-4,6-bis(dimethylamino)-1,3,5-triazine was chosen as difunctional starting material, whereat solubility in organic solvents is enhanced by the use of the N-alkylated product. Epoxidations of the allyl functionalities were carried out using several common epoxidation agents. Partially, conversion took place forming mainly by-products like substituted hydroxyl amines and hydroxy triazine derivatives. Nevertheless, epoxidation of double bonds took place forming different epoxy containing structures, which may be useful starting materials for further conversions.

## 1 Introduction

Justus von Liebig first prepared 2,4,6-triamino-1,3,5triazine (melamine) in 1834 by heating a mixture of potassium thiocyanate and ammonium chloride [1]. Since the development of melamine-formaldehyde (MF) resins in the 1930s [2], melamine has gained more and more industrial importance. Particularly, during the last years the use of formaldehyde for MF resins was of public interest, as the world health organization (WHO) classified formaldehyde 2006 as suspected carcinogen [3]. Thus, new possibilities for the cross-linking of melamine have been developed to replace formaldehyde. Nevertheless, until now no serious method has been found. An appropriate method to avoid formaldehyde is the synthesis of melamine derivatives bearing functional groups that can be cross-linked, for example vinyl groups [4] or epoxides. Pedroso et al. [5] already described the preparation of epoxy-modified melamine derivatives using melamine and epichlorohydrin. Thereby only the production of prepolymers was possible. Another approach for the synthesis of epoxides is the epoxidation of double bonds. Applicable starting materials for that purpose are allylmelamines, which are already known since 1948 [6]. By the reaction of cyanuric chloride with various amines, allyl substituted melamine derivatives bearing different functionalities are accessible. Recently, the polymerization of allylmelamines was investigated [7]. Epoxidation of these derivatives may lead to monomers that can be cross-linked by polyaddition forming new polymers. The aim of the presented work is the epoxidation of 2diallylamino-4,6-bis(dimethylamino)-1,3,5-triazine (1) using different epoxidation agents. On the one hand this starting material was chosen because it may lead to difunctional epoxides. Furthermore, the solubility in common organic solvents is increased using N-alkylated compounds. Established epoxidation agents like m-chloroperoxybenzoic acid (*m*CPBA), *tert*-butyl hydroperoxide (TBHP), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and sodium hypochlorite in combination with different catalysts were tested. Some reagents turned out to be too weak epoxidation agents, whereas others led to oxidation at which mainly by-products were formed, that will be presented in this paper.

## 2 Results and Discussion

The epoxidation of **1** using different epoxidation agents was studied, whereat next to the synthesis of 2-allylglycidylamino-4,6-bis(dimethylamino)-1,3,5-triazine (**2**) especially the preparation of 2-diglycidylamino-4,6-bis(dimethylamino)-1,3,5-triazine (**3**) was desired.

Scheme 1:



First, epoxidation was tested using TBHP. Because of its high selectivity and safety it is often applied in organic oxidations, activated by catalysts like  $MoO_3$  [8,9]. Attempts to epoxidize **1** were carried out in different organic solvents under reflux conditions. TBHP dissolved in water was used as well as anhydrous TBHP in decane (Table 1). Reactions

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were carried out with and without the addition of catalyst  $MoO_3$  and monitored by electrospray ionization-mass spectrometry (ESI-MS). Even after reaction times of more than 48 hours no conversion of **1** was detected. Thus, TBHP seems to be an inappropriate epoxidation agent for this purpose.

Table 1: Solvent systems used to epoxidize 1 using TB	HP
with and without addition of $MoO_3$ as catalyst	

			-	•
Entry	TBHP	Solvent	Catalyst	Result <sup>1</sup>
1	in water	tetrahydrofuran	no	-
1	in water	tetrahydrofuran	yes	-
2	in water	toluene	no	-
2	in water	toluene	yes	-
3	in water	chloroform	no	-
3	in water	chloroform	yes	-
4	in decane	chloroform	no	-
4	in decane	chloroform	yes	-

 $^{1} \pm$  = positive or negative conversion of starting material



Figure 1: By-products formed in the epoxidation of 1 using mCPBA: 2-hydroxy-4-diallylamino-6dimethylamino-1,3,5-triazine (4), 2-hydroxy-4-allylglycidylamino-6-dimethylamino-1,3,5triazine (5)

Epoxidation agents showing higher activities are peracids, like *m*CPBA. Prileschajew employed such compounds already 1909 in epoxidation reactions [10] and they are still used today. Therefore, **1** was reacted in dichloromethane at a temperature of 0 °C by stepwise addition of *m*CPBA. Analyses using ESI-MS showed signals probably referring to **2**, **1**, and by-products generated by hydrolysis of dimethylamino groups (**4**, **5**). Conversion could not be advanced by further addition of peracid.

The oil obtained after workup of the reaction mixture contained next to **1** about 76% mono-oxidized product, analyzed by high-pressure liquid chromatrography (HPLC-MS). However, NMR measurements showed that the formed product does not contain epoxy functionalities. The only change compared to the spectrum of **1** is the occurrence of two unequal signals of dimethylamino groups at 2.87 and 3.11 ppm (Figure 2).



Figure 2: <sup>1</sup>H-NMR spectrum of mono-oxidized product. CDCl<sub>3</sub> was used as solvent and internal standard. The signal at 3.06 ppm refers to unreacted starting material

Thus, oxidation occurred on a dimethylamino functionality leading to the formation of an amine oxide. The generation of amine oxides using *m*CPBA is well known [11,12], as well as the Meisenheimer rearrangement that can be performed using such compounds, whereas substituted hydroxylamines are formed [12,13]. Therefore, the formation of two different structures is possible (Scheme 2). Reaction may stop after generation of 2-dimethylamineoxide-4-diallylamino-6-dimethylamino-1,3,5-triazine (6) or a further rearrangement to 2-diallylamino-4-dimethylamino-6-(dimethylamino)oxy-1,3,5-triazine (7) occurs. Additional 2D NMR correlation experiments indicate the formation of 7, as no coupling between the protons of the oxidized dimethylamino group and the quaternary carbon atom of the triazine ring system was detected.

#### Scheme 2:



Table 2: Attempts to epoxidize **1** using  $H_2O_2$ , FeCl<sub>3</sub>,  $H_2$ pydic, and pyrrolidine

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Entry	Solvent	Result <sup>1</sup>
1	isopropyl alcohol	-
2	dichloromethane	-
3	dimethylformamide	-
4	acetonitrile	-

 $^{1} \pm =$  positive or negative conversion of starting material

Using *m*CPBA the generation of amine oxides is the preferred reaction. Therefore, the application of hydrogen peroxide as epoxidation agent was tested. As an experiment indicated, hydrogen peroxide alone is not able to epoxidize the double bond, but it may be activated using different catalysts. One possibility to activate peroxides is the use of FeCl<sub>3</sub> in combination with pyridine-2,6-dicarboxylic acid (H<sub>2</sub>pydic) and pyrrolidine [14,15]. Attempts to epoxidize **1** 

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using this system were carried out at room temperature using different solvents (Table 2). Reactions were monitored by ESI-MS, but even after reactions times of 24 hours only the unchanged starting material was detected.

Another method for the activation of hydrogen peroxide is the application of  $MoO_3$  as catalyst [16]. So, **1** was reacted with hydrogen peroxide and  $MoO_3$  in various solvents at different temperatures. Additionally, a few attempts were done using sodium hydrogen carbonate (NaHCO<sub>3</sub>) as promoter (Table 3). Except one experiment, all attempts did not lead to the formation of epoxy groups. Even using an excess of hydrogen peroxide no conversion took place. Only experiment 10 led to an oxidation of the starting material. As just this particular combination of solvent and catalysts is active, it is supposed that the reaction follows the mechanism shown in Scheme 3.

Table 3: Solvent systems used to epoxidize 1 using TBHP with and without addition of MoO<sub>3</sub> as catalyst

Entry	Solvent	Temperature	NaHCO <sub>3</sub> <sup>2</sup>	Result <sup>3</sup>
1	chloroform	r.t. <sup>1</sup>	no	-
2	chloroform	$40^{\circ}C$	-	-
3	chloroform	61°C	-	-
4	tetrahydrofuran	$40^{\circ}C$	-	-
5	tetrahydrofuran	66°C	-	-
6	tetrahydrofuran	r.t. <sup>1</sup>	+	-
7	$H_2O_2$	$60^{\circ}C$	-	-
8	acetonitrile	$40^{\circ}C$	-	-
9	acetonitrile	$82^{\circ}C$	-	-
10	acetonitrile	r.t. <sup>1</sup>	+	+
11	dimethylformamide	r.t. <sup>1</sup>	+	-

<sup>*l*</sup> r. t.= room temperature

 $^{2} \pm$  = addition/ no addition of NaHCO<sub>3</sub>

 $^{2} \pm$ = positive or negative conversion of starting material

Hydrogen peroxide is able to add on nitriles under light basic conditions forming peroxycarboximidic acids [17], which are stronger epoxidation agents than pure hydrogen peroxide.

#### Scheme 3:



An additional catalyst like  $MoO_3$  should not be necessary. Therefore, further experiments were done without catalyst using various amounts of hydrogen peroxide and NaHCO<sub>3</sub>. It was found, that conversion is higher when no extra catalyst is used and small doses of base are applied. Furthermore, several additions of small portions of peroxide and base led to better results, whereas beside **2** also **3** is formed.

Table 4: Attempts to epoxidize 1 using  $H_2O_2$  and acetonitrile

Entry	$H_2O_2$	NaHCO <sub>3</sub>	Result <sup>1</sup>
1	2x 10 eq.	<b>1</b> x 0.25 eq.	+
2	2x 10 eq.	<b>1</b> x 0.25 eq.	+
3	3x 10 eq.	<b>1</b> x 0.25 eq.	+
4	2x <b>5</b> eq.	1 x 0.25 eq.	+
5	3x 10eq.	<b>3</b> x 0.25 eq.	+
6	10 eq. + 2x <b>5</b> eq.	3 x 0.25 eq.	+

 $^{1} \pm$  = positive or negative conversion of starting material

The product obtained after workup was analyzed using HPLC-MS and contains next to 2 and 3 also by-products like 7. Differentiation between these products was done by comparing the fragmentations of the mass signals. However, the main components of the obtained solid are by-products like 5 and 8, which were isolated using column chromatography. The generation of epoxy functionalities was confirmed by NMR analyses (Figure 4). Furthermore, the mechanism via peroxycarboximidic acids was approved, as acetamide was isolated from the reaction residue. Epoxidation was successful, but the requested structures 2 and 3 were only formed in minor amounts. Basically, hydrolysis of dimethylamino groups occurred forming 4.5, and 8.



Figure 3: By-product formed in the reaction of 1with  $H_2O_2$  in acetonitrile. 2-hydroxy-4diglycidylamino-6-dimethylamino-1,3,5triazine (8)



Figure 4: <sup>1</sup>H-NMR spectrum of 5. CDCl<sub>3</sub> was used as solvent and internal standard.

A further attempt to epoxidize **1** was done using Jacobsen catalyst (Figure 5) [18,19]. Due to the high selectivity, this manganese based complex is often used in organic synthesis. As oxidant, usually bleach (aqueous sodium hypochlorite) is applied in combination with 4-phenylpyridine-N-oxide as co-oxidant in a two-phase sys-

#### tem of dichloromethane and water [20].



Figure 5: Jacobsen catalyst (9) used for oxidation

# 3 Conclusion

Epoxy modified melamine derivatives are interesting starting materials for the production of new polymers. In this project the synthesis of such compounds via epoxidation of an allylmelamine using various epoxidation agents was investigated. TBHP and bleach in combination with Jacobsen catalyst turned out to be unsuitable for this purpose. In contrast, the use of mCPBA induced oxidation, but not the formation of epoxides. Presumably, substituted hydroxyl amines were generated after Meisenheimer rearrangement of the developed amine oxides. Furthermore, hydrogen peroxide was tested in combination with different catalysts. The application of MoO<sub>3</sub> and FeCl<sub>3</sub> as catalysts was not successful. Epoxidation only occurred using a basic acetonitrile medium, whereat peroxycarboximidic acid is formed, that represents a stronger epoxidation agent. This reaction mechanism was confirmed by the analysis of acetamide, a side product only formed via this mechanism. Though epoxidation occurred, as affirmed by NMR analyses, mainly by-products formed by hydrolysis of dimethylamino groups could be isolated.

# 4 Experimental

All chemicals were of p.a. quality and used without further purification. Allylmelamine 1 was prepared by the reaction of cyanuric chloride with dimethylamine and diallylamine [6]. Melting points were measured using a Leica Galen III microscope with a Kofler melting point unit. ESI-MS analyses were done using a Thermo Finnigan LCQ Deca XPplus in positive ion mode. HPLC measurements were performed on a Thermo Electron Corporation Finnigan Surveyor MS Pump Plus, Autosampler Plus, PDA Plus Detector with Thermo Finnigan LCQ DECA XPplus mass detection. 1H, 13C, and 2D correlation NMR experiments were measured on a Bruker Avance DRX 200 MHz, a Bruker Avance III 300 MHz and a Bruker Avance DRX 500 MHz spectrometer using standard pulse sequences as provided by the manufacturer. FT-IR measurements were done using a Perkin Elmer Paragon 1000 PC FT-IR spectrometer and elemental analysis was done on a Thermo Scientific Flash EA 1112 CHNS-O Analyzer.

#### General procedure for TBHP

0.50 g (1.9 mmol) **1** and if used 1 mg (0.01 mmol) MoO<sub>3</sub> are dissolved in a three-necked round-bottom flask in 15 mL solvent. 1.0 mL (7.6 mmol) TBHP in water (70%) or

alternatively 1.4 mL (7.6 mmol) TBHP in decane (5.5 M) are added, whereupon the mixture is heated to reflux for at least 48 hours.

#### Epoxidation using *m*CPBA

In a three-necked round-bottom flask 0.50 g (1.9 mmol) 1 are dissolved in 3 mL dichloromethane and cooled to  $0^{\circ}$ C. Afterwards 0.66 g (3.8 mmol) mCPBA dissolved in 3 mL dichloromethane are added. Additionally, two equal portions of mCPBA are added in intervals of 3 hours. After a reaction time of 24 hours the mixture is neutralized using sodium hydroxide. The organic phase is washed three times with water, yielding 0.38 g of a yellow oil, containing 76% (HPLC-MS) of 7. ESI-MS (MeCN/H<sub>2</sub>O = 1:1):  $m/z = 279.1 [7+H]+; {}^{1}H-NMR (300 MHz, CDCl_3, 30 °C):$  $\delta = 5.83-5.76$  (m, 2H, -CH=CH<sub>2</sub>), 5.14-5.09 (m, 4H, -CH=CH<sub>2</sub>), 4.17 (s, 4H, >N-CH<sub>2</sub>-), 3.11 (s, 6H, >N-CH<sub>3</sub>), 2.87 (s, 6H, -O-N-CH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>, 30 °C):  $\delta$  = 171.7 (>C-O-N<), 166.7 (>C-N<), 166.2 (>C-N-allyl), 135.0 (>N-CH<sub>2</sub>-), 116.6 (-CH=CH<sub>2</sub>), 48.60 (-O-N-CH<sub>3</sub>), 48.17 (>N-CH<sub>2</sub>-), 36.38 (>N-CH<sub>3</sub>) ppm

#### General procedure for H<sub>2</sub>O<sub>2</sub> and FeCl<sub>3</sub>

In a three-necked round-bottom flask 25.8 mg (0.1 mmol) FeCl<sub>3</sub>x6H<sub>2</sub>O, 16.0 mg (0.1 mmol) pyridine-2,6-dicarboxylic acid and 16  $\mu$ L (0.2 mmol) pyrrolidine are dissolved in 20 mL solvent. After stirring the mixture for 20 minutes, 0.50 g (1.9 mmol) **1** dissolved in 2 mL solvent are added, followed by the addition of 0.78 mL (7.6 mmol) H<sub>2</sub>O<sub>2</sub> (30% in water).

#### General procedure for $H_2O_2$ , with and without $MoO_3$

0.50 g (1.9 mmol) 1, eventually 1 mg (0.1 mmol)  $MoO_3$  and 0.04 g (0.5 mmol) NaHCO<sub>3</sub> are dissolved in a threenecked round-bottom flask in 10 mL solvent. After 2.0 mL (19.1 mmol)  $H_2O_2$  (30% in water) are added dropwise, the reaction mixture is heated to the corresponding temperature. Additional amounts of peroxide and base are added in intervals of 1-2 hours.

#### Epoxidation using $H_2O_2$ and acetonitrile (attempt 6)

Attempt 6 is done using the sixfold amount, to obtain enough product for different purification steps. Excess of peroxide is neutralized by adding MnCO<sub>3</sub>, whereas insoluble MnO<sub>2</sub> is formed. After filtration, the organic phase is washed three times with water whereupon 0.60 g of a white solid are obtained. Further purification is done by column chromatography (silica gel 60, chloroform:methanol = 10:1) whereby three products are isolated. Fraction 1: 31 mg white solid of 4, mp: 130-135 °C; ESI-MS (MeCN/H<sub>2</sub>O = 1:1): m/z = 236.5 [4+H]+; <sup>1</sup>H-NMR (200 MHz, DMSOd6, 30 °C):  $\delta$  = 10.28 (s, 1H, -OH), 5.87-5.70 (m, 2H, -CH=CH<sub>2</sub>), 5.19-5.09 (m, 4H, -CH=CH<sub>2</sub>), 4.07 (s, 4H, >N-CH<sub>2</sub>-), 3.01 (s, 6H, N-CH<sub>3</sub>) ppm

<u>Fraction 2</u>: 0.14 g white solid mainly containing 5; mp: 184-185 °C; ESI-MS (MeCN/H<sub>2</sub>O = 1:1): m/z =252.2 [5+H]+; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>, 30 °C):  $\delta$  = 5.79-5.71 (m, 1H, -CH=CH<sub>2</sub>), 5.29-5.26 (m, 2H, -CH=CH<sub>2</sub>), 4.57-4.58 (m, 1H, CH oxirane), 4.00-3.98 (m, 2H, >N-CH<sub>2</sub>)

allyl), 3.95-3.92 (m, 1H, CH<sub>2</sub> oxirane), 3.78-3.65 (m, 1H, CH<sub>2</sub> oxirane), 3.69-3.65 (m, 1H, >N-CH<sub>2</sub>-oxirane), 3.30-3.28 (m, 1H, >N-CH<sub>2</sub>-oxirane), 3.12 (s, 6H, N-CH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>, 30 °C):  $\delta$  = 165.6, 159.0, 156.8, 131.1, 119.5, 64.20, 57.39, 46.80, 46.55, 37.41, 37.03 ppm; IR (KBr)  $\nu$  = 3365, 3238, 2971, 2927, 2872, 1675, 1601, 1561, 1517, 1462, 1393, 1285, 1140, 786 cm-1; elemental analysis for C<sub>11</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub> (%): C 51.27, H 6.78, N 27.15, O 12.55 (found), C 52.58, H 6.82, N 27.87, O 12.73 (calculated)

<u>Fraction 3</u>: 99 mg white solid mainly containing **8**, mp: 139-142 °C; ESI-MS (MeCN/H<sub>2</sub>O = 1:1): m/z = 268,2 [8+H]+; <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>, 30 °C):  $\delta$  = 4.66-4.58 (m, 1H), 4.01-3.74 (m, 4H), 3.51-3.20 (m, 2H), 3.21 (s, 3H), 3.16 (s, 3H), 2.85 (m, 1H), 2.61-2.58 (m, 1H) ppm; <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>, 30 °C):  $\delta$  = 165.7, 159.3, 156.8, 116.9, 64.29, 64.17, 57.71, 57.61, 49.90, 49.63, 48.43, 46.23, 44.82, 37.44, 37.04 ppm; IR (KBr)  $\nu$  = 3381, 3247, 2966, 2931, 1676, 1605, 1567, 1519, 1463, 1402, 1288, 1141, 1061, 786 cm-1; elemental analysis for C<sub>11</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub> (%): C 48.22, H 6.51, N 26.74, O 16.63 (found), C 49.43, H 6.41, N 26.20, O 17.96 (calculated)

<u>Acetamide</u>: 0.14 g white solid, mp: 78-79 °C, <sup>1</sup>H-NMR (200 MHz, DMSO-d6, 30 °C):  $\delta$  = 7.25 (s, 1H, -NH), 6.64 (s, 1H, -NH), 1.76 (s, 3H, -CH<sub>3</sub>) ppm; IR (KBr)  $\nu$  =3347, 3187, 2807, 2353, 2320, 1660, 1393, 1348, 1149, 1044, 1001, 873, 825 cm-1

#### Jacobsen epoxidation

At first a bleach solution is prepared by mixing 8.4 mL (12.5 mmol) sodium hypochlorite solution (10%) and 16.8 mL (0.05 M) of an aqueous disodium hydrogen phosphate solution. pH value is brought to 11.3 by addition of sodium hydroxide. A three-necked round-bottom flask is charged with 0.50 g (1.9 mmol) 1 dissolved in 2 ml dichloromethane, 0.13 g (0.8 mmol) 4-phenylpyridine-N-oxide and 0.08 g (0.1 mmol) 9 under an argon atmosphere. The mixture is cooled to 0 °C and 10.4 mL of the bleach solution are added over a period of 30 minutes. Stirring is continued for 5 hours. Afterwards, dichloromethane and water are added and the organic layer is washed two times with water. The black residue is further worked up using column chromatography (silica gel 60, chloroform:methanol = 30:1). However, neither product nor starting material was detected using ESI-MS and NMR.

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# **Open Questions**

#### Are Planck-Particles the Primordial Particles of Matter in the Universe?

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Received 25.08.2011, published 28.09.2011

# 1 The Values of the Constant of Gravitation and of Other Physical Constants

If one looks at the stars, it seems astonishing that their velocities as well as those of galaxies are negligible compared to speed of light after almost 14 billion years of gravitation and expansion of the universe. In fact, it seems that there is a very fine tuning between these two cosmic phenomena. It is therefore assumed, that "potential energy" caused by gravitation and "kinetic energy" caused by expansion of the universe are equal to each other (using the relations R = ctand  $E = Mc^2$ ):

$$\frac{GM^2}{R} = Mc^2 \rightarrow G = \frac{c^5 t}{E} \text{ or } G = \frac{R^5}{t^4 E} \qquad (1)$$

$$E = \frac{c^5 t}{G} \approx 1.3 \cdot 10^{70} J \text{ (for } t = 1.4 \cdot 10^{10} a \text{)}$$
 (2)

R: radius of the universe

c: velocity of light

t: age of the universe

G: constant of gravitation

M: mass of the universe

E: energy of the universe

Equation 1 has independently been proposed by Riofrio recently [1].

Similarly, one can obtain values for several other physical constants, using the equation above for G and the relations introduced by Planck more than hundred years ago [2]. For example, the value of the Planck constant h can be obtained by the following equation:

$$\Delta E \cdot \Delta t = N_p^2 \cdot \hbar \tag{3}$$

In this case,  $N_p$  is the number of "Planck-masses", expressed through division of the total energy of the universe by the Planck-energy:

$$N_p = \frac{E}{m_p c^2} \approx 10^{61} \tag{4}$$

By introducing equation 2 for  $\Delta E$  one obtains:

$$\hbar = \frac{c^5 t^2}{N_p^2 \cdot G} \tag{5}$$

Therefore, it seems that many if not all physical constants may be related to the structure (size, age and energy) of the universe.

# 2 The Energy of the Universe and Mass Production

Although very speculative, the following assumptions lead to conclusions, which are extremely surprising: The value of E in equation 2 is very close to recently published estimations for the whole energy of the universe [3]. It follows that either c [1] or G vary with the age of the universe (E= const.) or that E is a function of the age of the universe and increases linearly with the age (G and c remaining constant). If E was constant, then probably all other physical constants would have to vary, too, since they all seem to be related to each other [2]. Otherwise the "fine tuning" of

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them would be valid only for status quo, which seems to be most unlikely. Therefore, it seems to make more sense, to assume G to be a constant and E to vary with time, although this seems to contradict the principle of energy conservation. Introducing some kind of "negative energy" in the way

$$E + E_n = 0 \tag{6}$$

on the other hand, might be a solution for that problem (similar to  $\Lambda$  introduced by Einstein in his general theory of relativity). In this case, the principle of energy conservation applies to the sum of both "normal" and "negative" energy (however, any positive value of E, created in the big bang contradicts this principle, too!). It is interesting that in this case the total energy of the "system universe" would constantly stay null over time, thus needing no explanation for its existence.

Furthermore, the increase of positive (mass) and negative energy probably is not continuous, but happens in discrete steps. The smallest possible step for such an event may be the Planck-time:

$$\Delta t_p = \sqrt{\frac{\hbar G}{c^5}} \tag{7}$$

Using equation 2 in the form

$$\Delta E = \frac{c^5 \Delta t_p}{G} \tag{8}$$

and introducing equation 7 leads to

$$\Delta E = \sqrt{\frac{\hbar c^5}{G}} \,. \tag{9}$$

This is exactly the value of the Planck-energy, meaning that the increase in energy (or mass) of the universe in steps of the Planck-time is exactly one Planck-mass per time interval. Most curiously, this production rate gives exactly the observed mass of the universe after a total of about 14 billion years!

## 3 Cosmic Rays

The production rate of Planck-particles (one Planck-mass per Planck-time) deduced above can also be expressed as about  $10^{44}$  particles/second. Using the present volume of the universe (about  $10^{79} m^3$ ), this would give a value of about  $10^{-35}$  particles per  $m^3$  and second (or about 1 particle per 10 million years within the volume of the earth).

Particles of the cosmological radiation of very high energy interfere with the background of photons, thus losing parts of their energy. Therefore, particles coming from outside a distance of about 163 million light years should not exceed an energy of about  $6 \cdot 10^{19} eV$  (GZK-cutoff [4,5]). Nevertheless, several particles with higher energies have already been detected, meaning that they must have been produced within this radius.

One Planck-particle has enough energy to produce about  $10^9$  secondary particles with energies of about  $6 \cdot 10^{19} eV$ . Using the above deduced value of about  $10^{-35}$  Planck-particles per  $m^3$  and second, this would lead to a density of approximately  $10^{-26}$  particles per  $m^3$  and second, which seems to be in accordance with the observed values [6].

## 4 Conclusion

It has been demonstrated that probably all physical constants can be expressed in terms of cosmic parameters. Therefore it follows, that either all physical constants vary with time, which is contradicted by observation, or that the energy of the universe must increase with time. Since a production rate of one Planck-particle per Planck-time would exactly lead to the presently observed amount of matter in the universe, the question is posed, whether Planck-particles are still produced in our universe and whether they are the primordial particles of matter. Particles in cosmic rays exceeding an energy of  $6 \cdot 10^{19} eV$  may lead to an answer of this question.

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## **An Unexplained Observation**

### Which Intermediate Product Causes the Blue Coloring in a Grignard Reaction Involving Zirconium Chelates?

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Received 03.11.2011, published 03.12.2011

While working on the doctoral thesis about chelates of zirconium and hafnium – cf. [1] for preparation of the ligands and [2] for preparation of the chelates – we made an observation which could not be explained and therefore was only described in the doctoral thesis of Christof Tröltzsch, Leipzig, 1960, in the following way:

After adding 1 mmol tris(1,3-diphenyl-1,3-propanedionato)-zirconium chloride to a solution of 10 mmol cyclopentadienyl magnesium chloride [3] in dry diethyl ether under dry pure nitrogen at room temperature, an intensive green color was immediately observed, which later changed to dark brown under evolution of minute gas bubbles. At room temperature, the same observations were made for the reaction with ethyl magnesium bromide.

At -20°C the green color appeared slowly. At temperatures below -5°C the green color was stable for more than 24 hours under inert conditions. When the temperature rose to 0°C or higher, the brown color appeared under gas evolution. Phenyl magnesium bromide showed the same behavior. A similar phenomenon was observed in the reaction of ethyl magnesium bromide with tris(1,3-diphenyl-1,3-propane-dionato)zirconium bromide, tetrakis(1,3-diphenyl-1,3-propanedionato)-zirconium, tetrakis(1,2,3-triphenyl-1,3-propanedionato)-zirconium, and tetrakis(1,3-bis(*p*-biphenyl)-1,3propane-dionato)-zirconium, but in these cases the color was an intensive blue-green.

We suspect that the color of the unknown reaction product is blue and that the blue-green shade is the result of a mixture with yellow chelates. In some cases the blue-green color changed via yellow to brown when heated to temperatures above 0°C. An analogous reaction under the same conditions with tetrakis(2,4-pentane-dionato)-zirconium at -15°C instantaneously showed a yellow color, which then changed to brown. No green or blue-green intermediate coloring was observed.

We suppose that the blue color is caused by the reduction of zirconium(IV) to zirconium(III), which is only stable when the unpaired electron of zirconium(III) is stabilized by conjugation of the chelate ring with aromatic systems. Further stabilization can be achieved by voluminous aromatic substituents.

All experiments were conducted in a flask, which priorly had been used to prepare the Grignard reagent, thus unreacted magnesium was still present. Besides the Grignard reagents the magnesium could also act as reducing agent.

B. N. Chakravarti [4] prepared tris(2,4-pentane-dionato)titanium which dissolves in benzene with intensive blue

color. Titanium dihalides and titanium trihalides are much more stable than the corresponding zirconium and hafnium halides [5-8]. This explains why a titanium(III) chelate only slowly reacts with oxygen, while a zirconium(III) chelate is solely stable under inert conditions, low temperature, stabilisation by mesomerism and perhaps steric shielding.

We tried to produce the corresponding chelate from zirconium trichloride – prepared according to [5] – and 1,3-diphenyl-1,3-propane-dionato sodium, but the reaction failed. The preparation of 1,3-diphenyl-propane-1,3-dion itself does not lead to the desired result, because the released hydrogen chloride immediately reacts with zirconium trichloride, which results in zirconium tetrachloride and hydrogen.

To test the magnetism of the unknown intermediate, a sample of the green solution, which was as concentrated as possible and prepared by the reaction of ethyl magensium bromide with tris(1,3-diphenyl-1,3-propane-dionato)-zirconium chloride in diethyl ether, was filled into a Quinke tube [9] with cooling jacket and put into an inhomogeneous magnetic field of max. 20000 Gauss. All procedures were carried out well below 0°C. The solution showed diamagnetism, but an approximate calculation indicated that the diamagnetism of the solvent can be dominant.

We suppose the zirconium(III) chelate with 1,3-bis(*p*biphenyl)-1,3-propanedione to be most stable, but this ligand will not be commercially available and should be prepared according to Ref. [1]. We think that ESR measurements could solve the introductory question. Despite my habilitation in 1967 I was not free in deciding the direction of my research activities and after 1990 other work was much more important. I would be very glad if someone in the scientific community could solve this old problem.

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