Michael W. Linscheid

Interactions between Biosphere and Environment

Analysis of Chemically Modified DNA

The food we eat, the air we breathe, the things that surround us, and the drugs we take to overcome our illnesses – they all contain natural or man-made compounds which can interact with our cells' building blocks. Most of these reactions are essential to life and follow the paths nature worked out and optimized over the centuries in order to guarantee our survival. This is valid, too, for traditional and modern drugs as well as artificially developed compounds, with the difference that nature enabled our body to identify new and unknown structures, and to deal with them. In case the compounds are useless or even dangerous, certain processes will try to remove them as soon as possible. The compounds will be changed by chemical reactions and made water-soluble, so that they can be eliminated. If this process fails or is too complicated, intermediate compounds can form which react with formerly not attacked parts of the cells, for example the DNA.

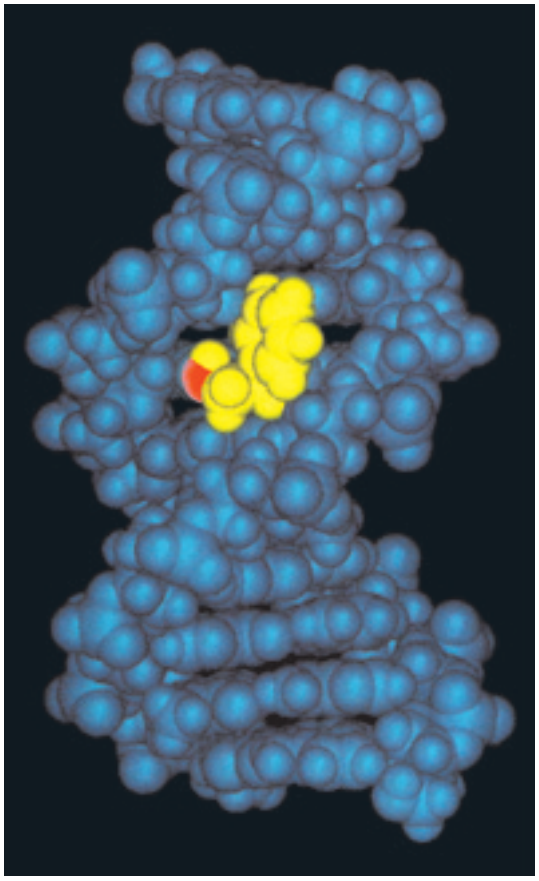


Fig. 1
Three dimensional structure of DNA strand with styrene oxide modification
CPK representation of the final MD structure for the S(12,1) duplex. Styrene oxide is shown in yellow (for details see ref. [1])

But nature has foreseen even this and provides mechanisms and possibilities able to correct and repair useless and dangerous changes before they can lead to irreparable damages. So, our biological processes are sturdy and tolerant against chemical and biological divergences, stabilizing life within completely different environmental patterns, and giving us the means to protect ourselves against perturbations. Only if substances bypass or break through all these protective (and other biological) measures, chemical reactions can provoke changes leading to biological consequences, that means to the formation of tumours or cancer.

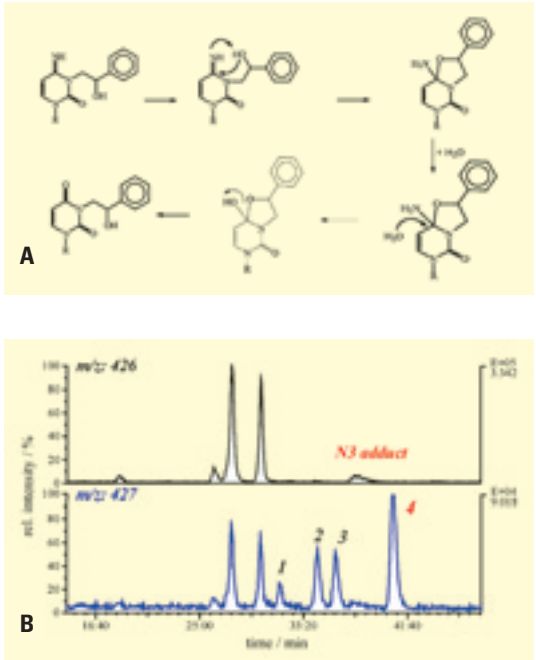
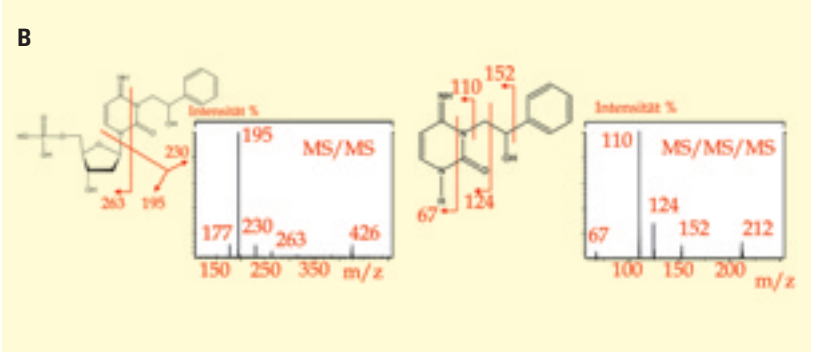


Fig. 2
A) Reaction of styrene oxide with cytosine; in the course of the reaction a deamination takes place, changing the nucleobase from cytosine to uracil
B) HPLC separation of the cytosine and uracil adducts (top) and fragmentation and MS/MS data (bottom) [2].



The impact on analytical chemistry is twofold: First, the analytical chemist is confronted with problems which often require new methods and new technical processes. Second, one has to be conscious that we can elucidate only small parts of the complex chain of events by chemical methods. Our methods of investigation cannot explain everything, and we need to cooperate with biochemists, biologists and physicians if we want to extend our knowledge.

In our group one of the major areas of research is focused on reactions between compounds entering the body and DNA. Our main objective here is to develop tools, which enable us and others to handle problems better and more efficiently. We want to try out these tools, use them for projects where colleagues from different disciplines cooperate, and finally hand them on to others.

Reactions between chemical compounds and DNA involve many problems. In fact, we have to make the assumption that chemical changes to DNA are normal

and probably numerous, but how and to what extent these natural (endogenous) modifications occur is yet not well known. So, we should first ascertain what the naturally occurring changes to DNA look like, how numerous they are and of what structure.

Next, we should determine which kind(s) of compound(s) can undergo reactions, or better: Which compounds can be changed (metabolized) within the body so that these metabolites can react with DNA? The way through the cells up to the DNA is long, and a lot of other possible partners for reaction have to be bypassed before. (Fig. 1)

Then, we have to investigate the chemical structures forming through exogenous processes (substances entering the body from outside), and their behaviour: Are the changes stable (long-lived) or unstable, are the substances converted or can the changes be reversed? Often not only adducts, but also many other products are formed, that means, the relative and absolute numbers and quantities have to be determined. These are the preliminaries which help us answer the question: Are the occurring changes of any consequences or not?

Wechselwirkungen zwischen Biosphäre und Umwelt

Reaktionen von Stoffen in unserer Umwelt mit biologischen Makromolekülen können zu Strukturen führen, die in den Zellen nicht gewollte Folgereaktionen auslösen. Derartige Reaktionen an DNA beschäftigen uns nun schon seit mehreren Jahren, wobei die Entwicklung der dazu notwendigen analytischen Verfahren oder auch die Verbesserung von vorhandenen Methoden einen wichtigen Teil der zu leistenden Arbeit darstellt.

Wir optimieren aber nicht nur solche Verfahren, sondern versuchen auch, sie – zum Teil in Kooperationen mit Biochemikern und Medizinern – auf Fragen anzuwenden und Antworten zu finden, die es erlauben, in Zukunft genauere Informationen zu den chemischen Grundlagen von Veränderungen in biologischen Systemen zu erhalten. So untersuchen wir DNA-Addukte von xenobiotischen Stoffen in der Umwelt ebenso wie solche in Arzneimitteln. Wichtige Werkzeuge sind dabei die Chromatographie, vor allem als Mikro- oder sogar Nano-Chromatographie gekoppelt mit den modernen, extrem leistungsfähigen massenspektrometrischen Techniken wie Elektrospray oder ICP.

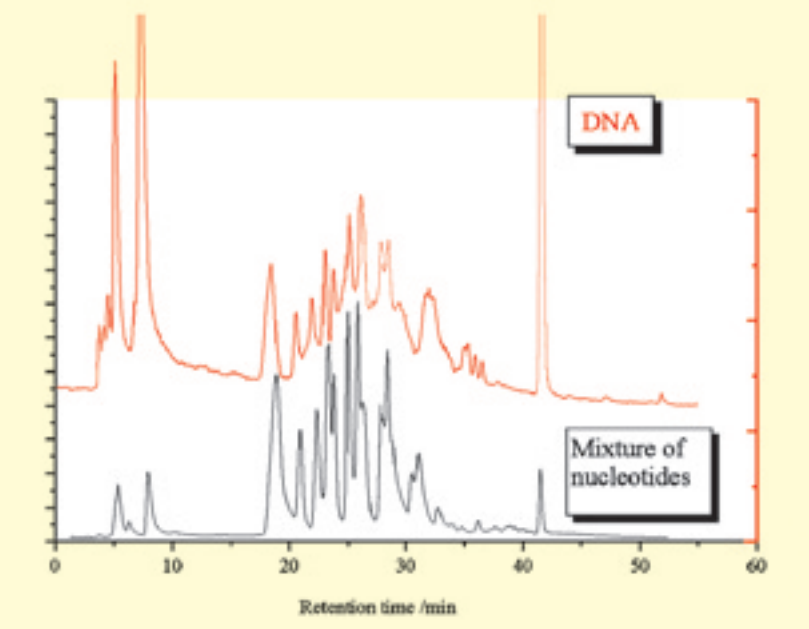
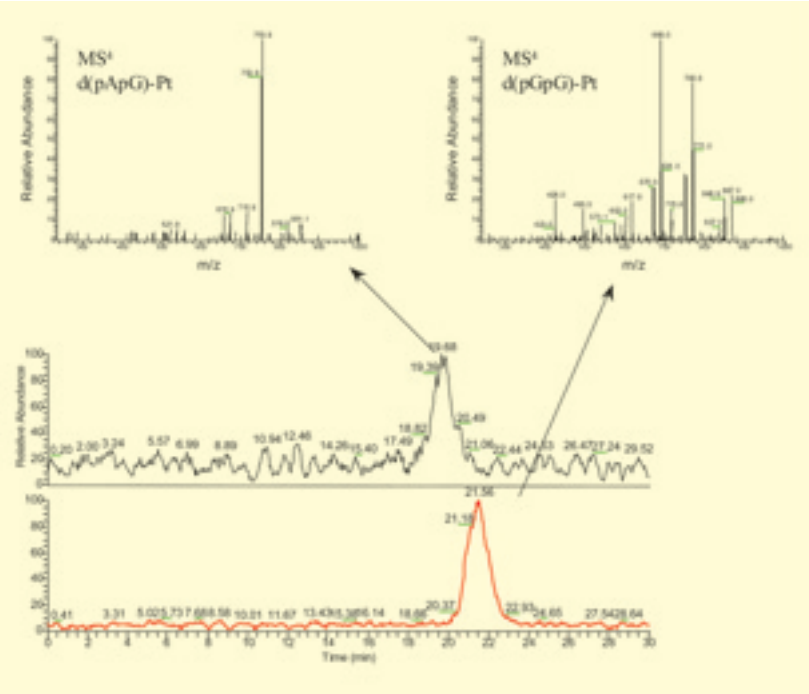


Fig.3
Detection of modified Nucleotides using ICP MS; the signal for phosphorus (m/z 31) is used for detection. An internal standard for quantification is added to the separation (last peak, bis-nonyl-phosphate) [5].

Fig.4
Adducts of cis-Platin to the dinucleotides d(pApG) and d(pGpG), after separation using HPLC and identification with ESI MS^4 [6, 7].



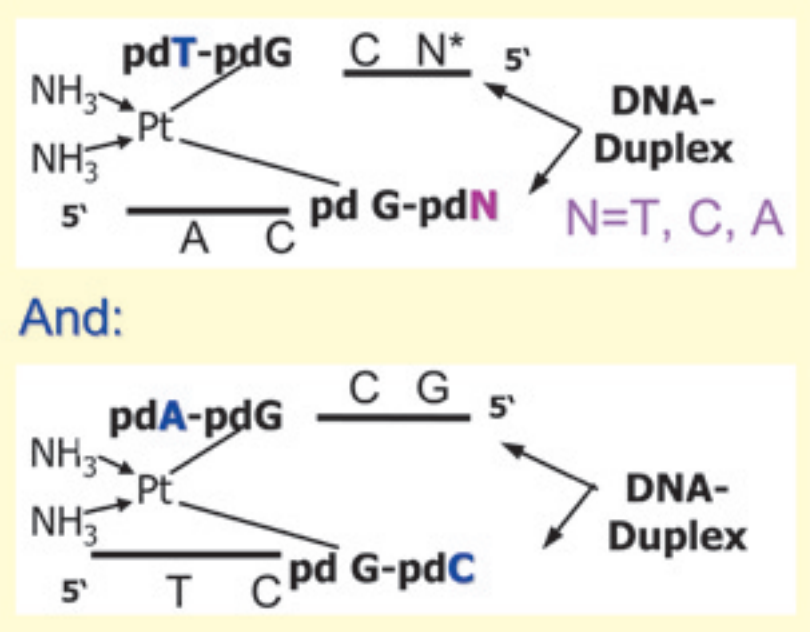
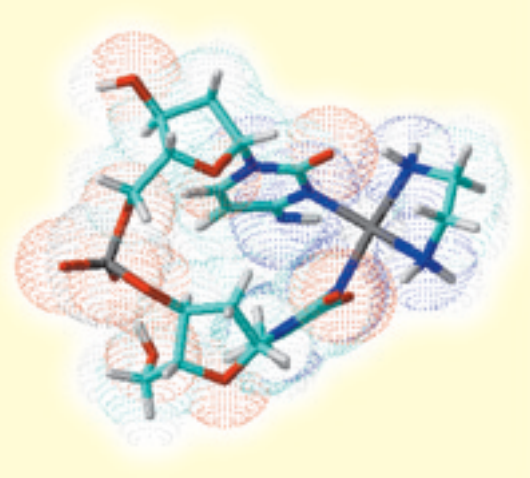


Fig. 5
Cartoon of adducts of cis-Platin to guanosin (G) in DNA allowing to locate the site of reaction within the DNA duplex and possible nucleobases in the neighbourhood [8].

By and large, it is not easy to get toxicologically meaningful results, even if recent developments permit in some cases to gain new information. Using extremely low labelled ^{14}C containing products and detecting them by accelerator mass spectrometry may possibly lead the way to dosimetry and help clear up the question: Was an adduct formed and how much of a given substance? Until now, the presence of an adduct has been determined by the most effective »post-labelling method«, which unfortunately is not easy to handle because of the necessarily great amount of radioactive radiation and because of some quantitative disadvantages, especially the impossibility to shed light on unknown structures. One can tackle now these difficulties using the methods offered by mass spectrometry, especially when coupled with micro separation techniques. GCMS has been used very successfully with some DNA adducts, but limitations are imposed by chromatography, which works only with compounds relatively stable against temperature changes.

Fig. 6
Structure of Pd ethylenediammine-CpT complex; ab inito calculation using GAMESS version 6.2, 2001 [9].



Our efforts were concentrated to study the new structures formed in reactions of platinum and palladium complexes, UV light and metabolites of styrene oxide as a test case for the power of the developed strategy. Styrene oxide, the main metabolite of styrene in the body, is known to react with DNA components and many different products can be formed. We have used an online combination of micro HPLC and Ion Trap mass spectrometry to separate and identify more than 40 different adducts in varying amounts. It can be shown that the reaction not only adds the styrene oxide molecule to a nucleobase or the phosphate, but reactions take place which change the chemical nature of the base itself e.g. from cytosine to uracil or adenine to xanthine. (Fig. 2)

Due to the great number of adducts it is almost impossible to quantify the products using similar compounds as internal standards. Therefore, a different approach was developed based on phosphorus. ICP MS, a mass spectrometric technique based on an inductively coupled plasma (=ICP) is a very powerful element detection technique. The plasma destroys organic compounds almost independent from its nature into the elements thus any phosphate can be used as internal standard to quantify unknown compounds which have a phosphate group. We did this successfully for the nucleotides [3] and later the same approach was employed for peptides as well [4]. (Fig. 3)

ICP MS was developed for the detection of metals, thus metal adducts to DNA can be detected at very low concentrations as well. We have used this to search for platinum adducts to DNA and we have found new, hitherto unknown adduct after reaction of the drug cis-Platin with DNA and subsequent enzyme digest.

Recently we could solve the nature of the new adducts using electrospray Ion Trap MS. We found in addition to the well known adducts of cis-platin to guanosine and adenine in dimeric structures (see Fig. 4) longer oligomers revealing the neighbouring bases in the DNA stand to the reacted bases. Even though mass spectro-

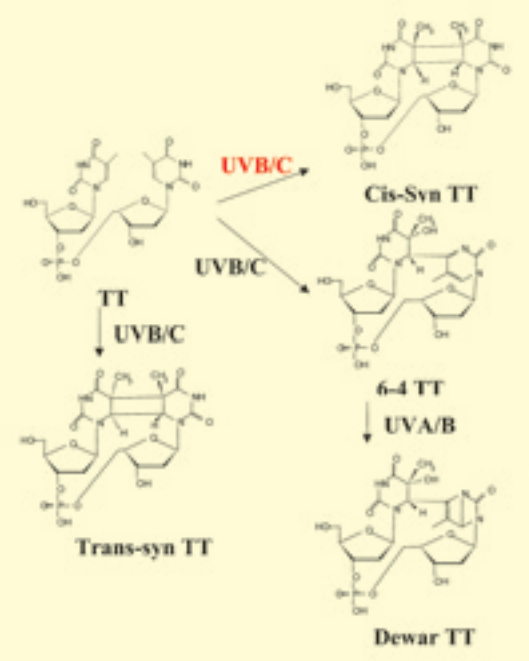


Abb. 7
Possible UV induces TpT lesions [11].

metry does not allow solving any detail in the course of the reaction, the assignment of some of the products was possible with only a few assumptions. Those assumptions appear justified due to the knowledge previously collected. The figure shows a summary of the structures found after separation on a RP micro column and a series of MSN experiments. (Fig. 5)

Recently we have palladium complexes to mimic the platinum reaction on faster time scale. The complex between ethylenediamin-palladium(II) is formed almost instantaneously and its reaction in the gas phase as currently under investigation; the calculated structure is shown in Fig. 6 and we currently investigate the relation between its fragmentation reactions and the structure based on the modelling results.

Finally a project shall be introduced which is aimed at the reactions in DNA under the influence of UV light, which may eventually, induce skin cancer. It is known that depending on the energy of the light either direct lesions in DNA or lesions via reactive radicals can be formed. Pyrimidine dimers are a major class of photo damage caused by UVC/B. UVA will lead to mainly oxidative DNA damage. Both types of lesions have received increasing attention with respect to their roles in mutation and skin cancer. In order to study their biological effects, sensitive analytical methods need to

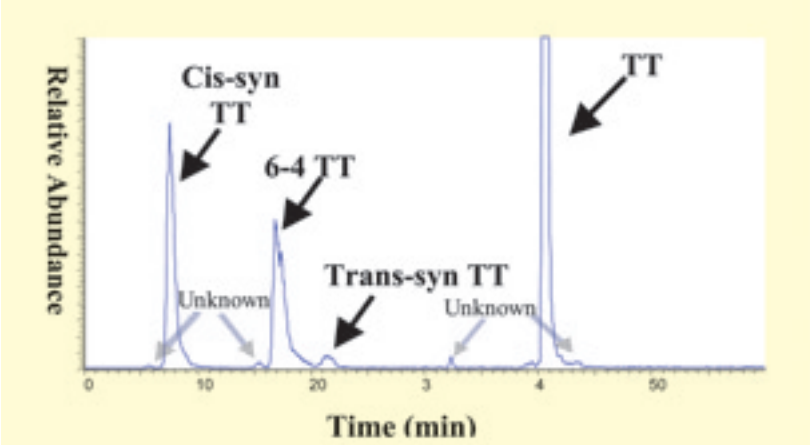
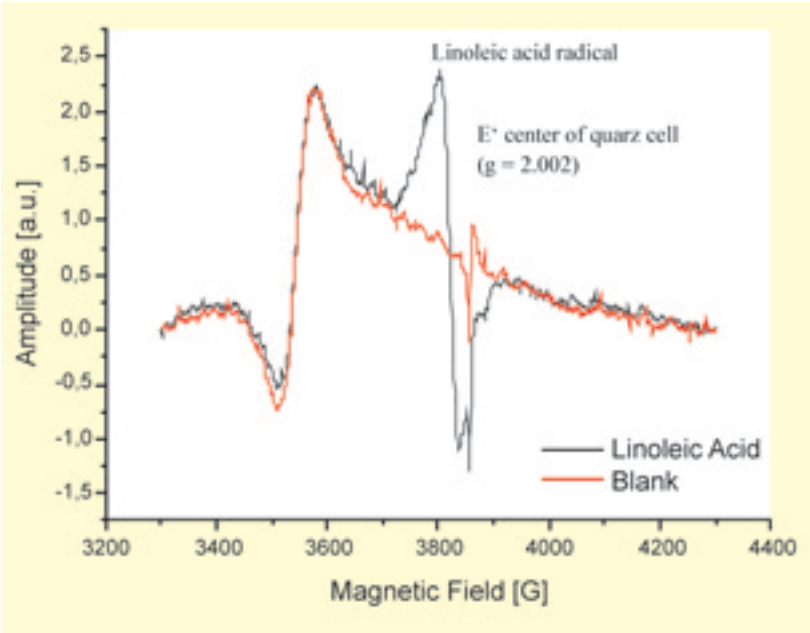


Abb. 8
Chromatographic separation of the photoproducts of TpT on a micro column. DNA was irradiated with UVC (254 nm), then digested using Nuclease P1 and Phosphatase; for the separation a RP phase has been used and an Ion trap mass spectrometer (FINNIGAN Ion trap LCQ Deca) for detection [11].

be developed. We have used micro-HPLC to separate the photoproducts of Thymine, including cis-syn TT, trans-syn TT, and 6–4 TT in UVC irradiated model compound TT and calf thymus DNA (Fig. 7). Then the structures were identified based on their MS/MS spec-

Fig. 9
Linoleic acid (pure): irradiated with UV-Light (365 nm) for 47 min at 77 K. The signal is increasing with time. Standard: $CuSO_4$ [12].



Prof. Dr.
Michael W. Linscheid
Born 1948. Diplom chemist 1973 (University of Cologne), Dr. rer. nat. 1975, 1980 Postdoc (with Prof. A.L Burlingame, Berkeley, CA, USA), 1981 Head of the working group »Organic Analysis« at the Institute of Spectrochemistry and Applied Spectroscopy ISAS, Dortnund; 1998 Prof. of Analytical Chemistry, Humboldt-Universität zu Berlin; 1989–92 Chairman of AGMS; 1993 Science and Technology Agency Fellow (STA Fellowship), Japan, Habilitation 1995, Dean of Faculty of Mathematics and Natural Sciences I (since 2002); Editor »Journal of Mass Spectrometry«.

Contact
Humboldt-Universität
zu Berlin
Faculty of Mathematics
and Natural Sciences I
Department of Chemistry
Brook-Taylor-Str. 2
D–12489 Berlin-Adlershof
Phone: +49 30 2093–7588
Fax: +49 30 2093–6985
E-Mail: michael.linscheid@
chemie.hu-berlin.de

tra of negative ions using ion-trap mass spectrometry [10] (Fig. 8).

In addition, 8-Oxo-deoxyguanosine, the most important oxidative DNA damage from UVA treated DNA samples, is under investigation as well. The goal here is the quantitative correlation between the number of radicals formed in the primary UV reaction and the amount of damage following this as consecutive chemical modification. The sequence is known to start with OH-radicals, which induce carbon centred radicals in membranes and skin compartments. As an example in this context linoleic acid is studied and in figure 9, the EPR signal of the radical is shown at 77 K. (Fig. 9)

The examples given here serve only to demonstrate that the relevance of the analytical methods developed in our group is embedded into questions arising from biology and medicine and the interactions with the environment. The future work will be aimed at the ever ongoing quest for smaller amounts in increasingly complex matrices. Only then the biological relevant processes can be reached and chemically understood. This is the field, as we see it, based on analytical chemistry, but with links into a wide range of different sciences, from physical chemistry to bioorganic chemistry and from biology to medicine.

A great number of co-workers were in the past and still are involved into the work behind the results presented here, namely Christoph Siethoff, Norbert Jakubowski, Wolfgang Schrader, Guangyu Zhang, Ulrike Hochkirch, Zhihua Wang, Michael Edler und Timo Hagemeister.

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