**Applied
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REVIEW

Möbius-Strip Topology of Expanded Porphyrins: A Minireview on EPR, ENDOR and DFT MO Studies

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Abstract

The one-sided Möbius strip with its characteristic 180° twist in the loop has inspired philosophers, artists and scientists since hundreds of years and continues to do so. On the molecular level, only in the last 15 years have some groups succeeded in synthesizing new expanded porphyrin compounds large enough to adopt Möbius-strip topology and Möbius aromaticity, the first being Lechosław Latos-Grażyński and collaborators in Wroclaw (2007) and Atsuhiro Osuka and collaborators in Kyoto (2008). We report on new studies of expanded porphyrins with either Möbius topology or Hückel topology that were synthesized in these laboratories. In this minireview, we focus on recent continuous-wave and time-resolved EPR, ENDOR and DFT MO studies on open-shell states of di-*p*-benzi[28]hexaphyrin(1.1.1.1.1.1), specifically, on the ground-state radical cation doublet state (total electron spin $S = 1/2$) and the first excited triplet state ($S = 1$). The review is largely based on the results and discussions of two previous publications: Möbius et al. (Appl Magn Reson 47:757–780, 2016) and Ema et al. (J Phys Chem Lett 9:2685–2690, 2018). In the open-shell systems, besides the electron-nuclear hyperfine couplings also the zero-field interaction tensor turned out to be a viable sensor for electronic structure changes between Möbius and Hückel topologies. In the Outlook section, we address the cyclotides, a new class of natural circular mini-proteins, usually less than 100 amino acids long. They are distinguished by exceptional chemical and biological stability. This is due to topological constraints imposed by *threefoil* knot and Möbius-strip formation. As a result, their physical qualities are "topologically protected". This makes them highly interesting for medical or agricultural applications, for example as novel active ingredients against autoimmune diseases, viral infections, or as agents against insect damage to crops.

Dedicated to Professor Takeji Takui (on the occasion of his 80th birthday).

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1 Introduction

The "Möbius strip" is a loop-shaped topological figure with a half-twist (180°) in its ribbon which results in a one-sided surface. It was topologically analyzed first by the nineteenth century German mathematicians August Ferdinand Möbius (1790–1868) and Johann Benedict Listing (1808–1882). For many centuries, the amazing symmetry behavior of half-twisted closed ribbons played—and still plays—a remarkable role in Western and Eastern cultures as a symbol of unity despite diversity.

It also is a symbol of our on-going connectedness and interaction, regardless of any differences in our individual movements. Even if we start walking on the Möbius strip from different points and in different directions, we will ultimately meet on the same side. In modern times, the Möbius strip is sometimes also considered as a symbol of an optimistic view of the future of our globe: despite all the differences in national interests and priorities, and despite almost insurmountable disagreements in the world about the right path to prevent an impending climate catastrophe, in the end, we will succeed in moving forward together on the one-sided path of fact-based, reason-driven science.

Such an optimistic view is highly needed at the present time, when destructive forces are grasping for power around the world, working with Fake News and rejecting the pursuit of Truth. But what we really need is free and open science. This is a prerequisite for scientific research to increase our knowledge of effective tools for urgent problem solving—while remaining trustworthy to the public.

Actually, in our essay the one-sided Möbius strip is the connecting element between the EPR work on expanded porphyrins of the Japanese research team in Sendai (Seigo Yamauchi), Osaka (Takeji Takui, Kazunobu Sato), Kobe (Yasuhiro Kobori) and Kyoto (Atsuhiro Osuka), having started on the Eastern side of the globe, and the EPR work on expanded porphyrins of the German–Polish research team in Berlin (Klaus Möbius, Martin Plato), Mülheim (Wolfgang Lubitz, Anton Savitsky), and Wroclaw (Lechosław Latos-Grażyński, Marcin Stępień), having started on the Western side of the globe. But both teams met on the one-sided path to the scientific advancement of knowledge, because they were using the same twisted expanded Möbius [28] hexaphyrin porphyrin molecule as target of their quite different EPR spectroscopic and quantum chemical investigations.

Hence, we regard the Möbius strip granite sculpture by Kaizo Ushio "Dream lens", 2003, at the Akashi-Kaikyo Bridge in Kobe as an enigmatic symbol for our transboundary topological studies (see Fig. 1). Keizo Ushio is one of Japan's most highly regarded sculptors and renowned internationally for his extraordinary granite sculptures.

With a span of 1991 m, the Akashi-Kaikyo Bridge is the longest suspension bridge in the world—and a source of pride for Japan. It connects, with its six freeway lanes, the Tarumi-ku district of Kobe on the main island of Honshu with the town of Awaji on the southern island of Awaji-shima. Thereby, it provides a network of connectivity between the centers of the Osaka-Kobe-Kyoto region which had experienced an extraordinary economic and cultural growth between 1950 and 1970.

Fig. 1 Kaizo Ushio, Möbius strip granite sculpture "Dream lens", 2003, public commission for the Akashi-Kaikyo Bridge in Kobe (Japan). The Akashi-Kaikyo Bridge (opened 1998) is the embodiment of half a century of technological progress in Japan. The Möbius strip sculpture is made of rock with three different types of surface, it represents people, nature, and science. It also represents the main island of Japan, Awaji Island, and Shikoku, which are now connected by the Akashi-Kaikyo Bridge. The three surface layers of Kaizo Ushio's Möbius strip sculpture harmonize well and express the hope for a harmonious future. Source: Hyogo Parks and Horticulture Association Maiko Park management office, Kobe-City

It is this region where most of the Japanese teams featured in this article are based. At this point in our story, it is sad to recall that our dear friend and highly esteemed colleague Seigo Yamauchi from Tohoku University in Sendai had passed away in September 2012. At a much too early stage of his life. We remember with deep gratitude his relentless support for the establishment of a firm cooperation and friendship between the Sendai and Berlin EPR laboratories. It is said that "*The only dead are those who are forgotten*". Seigo Yamauchi will not be forgotten, but will continue to be with us.

Those of us who attended the 2nd International Symposium on Electron Spin Science in Matsushima (near Sendai), July 23–25, 2012, vividly remember Seigo Yamauchi who served as Chairman of that Symposium. He was very dedicated to making this international meeting happen—only 1 year after the horrendous Tohoku earthquake and tsunami (March 11, 2011) with inconceivable destruction and human sacrifices. With the subsequent explosion of the Fukushima nuclear power plant, followed by large-scale radioactive fallout and contamination of the environment. According to official figures, more than 230,000 people were evacuated, living far from their homes, either in temporary lodgings or permanent resettlements. With the 2nd International Symposium on Electron Spin Science in Matsushima, Seigo Yamauchi wanted to express that Japan has risen again after these devastations—and looks to the future with confidence.

The days we spent as symposium participants in Matsushima and Sendai will remain unforgettable for us. We are grateful for the overwhelming hospitality by Seigo Yamauchi and his Japanese colleagues during this time, in particular by

Takeji Takui, Noboru Hirota, Masamoto Iwaizumi, Yasunori Ohba, Kimio Akiyama, Hideto Matsuoka, Yasuhiro Kobori, just to name a few.

Only 2 months after the Symposium, on September 26, 2012, Seigo Yamauchi passed away unexpectedly. What a shock! He had only 68 years to live! He still had so many plans he wanted to implement! In commemoration of late Seigo Yamauchi, Takeji Takui wrote in a collection of obituaries published in *EPR Newsletter* (Vol. 22, November 2012):

> *"I would like to say that Seigo was a man who knew the most about molecular excited states," characterizing salient features of Seigo as an outstanding scientist. In order to show how immense Seigo's scientific achievements and his life work are, I would like to say: "When you feel a sudden gust of wind blown through science, Seigo will be around. Seigo will never be a past scientist, but live as thousands of winds, as long as excited states are kept studied in science. ... I believe that life is short but science is long like art. Seigo's achievements will last with his name in the scientific world."*

Takeji Takui and his colleagues Yasuhiro Kobori (Kobe University) and Atsuhiro Osuka (Kyoto University) and their teams were very engaged in not letting the scientific contributions and footprints of Seigo Yamauchi be forgotten. In 2018, they published a seminal paper on excited triplet-state hexaphyrin molecules of Möbius strip topology. Thereby, they completed a cross-Japan collaboration which dates back to 2009. Takeji Takui and his coworkers, for instance, were involved in heavy quantum chemical MO calculations on the electronic structures in the lowest excited triplet states of those expanded porphyrins [1], see below.

When we were reflecting on our contribution to the special issue in honor of Takeji Takui's 80th birthday, we were tempted to write a Haiku poem about his work over the past decade or so. This work has much to do with quantum computing strategies, including those using multiple spin systems with nontrivial Möbius topology. We hereby dedicate our Haiku to Takeji:

Takeji's spin pool.

Möbius topology.

Qubits near and far.

(Just to clarify: Haiku poems have become very popular in Japan since the end of the seventeenth century, later they have enjoyed great appreciation also in Western cultures. Haiku is said to represent the world's most compressed poetry. A traditional Haiku consists of verses of three groups of words with five, seven, and five Japanese syllables: 5-7-5, for a total of 17 syllables. The entire universe in 17 syllables!).

K. M.'s and M. P.'s first encounter with Takeji Takui dates back to May 1977. It happened in Banff, Alberta, Canada, high up in the spectacular Canadian Rocky Mountain Park, at the occasion of the 6th ISMAR meeting, organized by Daniel Fiat, Chicago, then Chairman of ISMAR (International Society of Magnetic Resonance). It was a very memorable meeting insofar as, according to the

scheduled program, ISMAR wanted to honor the 1944 discoverer of EPR, Evgenii Konstantinovich Zavoisky from Kazan, USSR, with an international prize of high reputation in the magnetic resonance community. Tragically, Evgenii Zavoisky could not personally come to Banff to receive the Award. He had died (1976) in Moscow at the age of only 69. Hence, the International Society of Magnetic Resonance had to posthumously confer the ISMAR Award for the year 1977 on Evgenii K. Zavoisky (1907–1976) "in recognition of his discovery of the electron paramagnetic phenomenon in Kazan, USSR, in 1944". The Award Certificate was signed by 1966 Nobel laureate Alfred Kastler, Chairman of the Prize Committee, and Daniel Fiat. Karl H. Hausser, Heidelberg, who had nominated Zavoisky for the ISMAR Award, gave the posthumous Award Address. It was in fact very moving. He said:

"We are here in memory of a great scientist and his important discovery, Professor E. K. Zavoisky and electron paramagnetic resonance. … The full impact of Zavoisky's discovery together with the independent discovery of nuclear magnetic resonance by Purcell and Bloch becomes clear at this meeting, to which, 30 years later, so many scientists—physicists, chemists, and biologists—have come together from many countries in order to discuss the different aspects of magnetic resonance."

But sadly without him. In his memory, there exists now the prestigious Zavoisky Award which is annually granted by the Zavoisky Physical-Technical Institute in Kazan together with the Kazan Federal University.

Takeji Takui remembers (in a letter of October 9, 2017, responding to K. M.'s congratulations for having been honored by the 2017 Zavoisky Award) 40 years after our first encounter in Banff:

"In my career in terms of being a scientist, I have encountered many distinguished scientists after graduated from Osaka University. They have influenced my scientific career always strongly. I cannot list all of them nor describe all what such influence looked like. But what happed at Banff ISMAR Conference in 1977, the first international conference I have ever attended as a postdoc, has still been unforgettable. For the first time, I together with a young German postdoc met Klaus Möbius and his coworker Martin Plato at the bar of the Conference Center just by chance. Young Klaus … asked us what our topics were, listening to us very carefully, giving his scientific comments, and eventually hoping future success for both of us (to our surprise, he kindly bought beer for the two of us). Klaus really treated us just like independent and established scientists."

In the subsequent decades, Takeji Takui and Klaus Möbius, together with their respective coworkers in Osaka and Berlin, were orbiting separately around their common center of gravity, which was magnetic resonance. But they were meandering in different sub-worlds of EPR spectroscopy, namely "spin chemistry". This field, which is positioned at the intersection of photochemistry and photophysics, is characterized by weak interactions in and between the reaction

partners—but with major consequences for the reaction paths taken. Their work was (and is) focused on magnetic resonance studies of reaction intermediates and products of free-radical and triplet-state chemistry. They observe spin-polarization phenomena like CIDNP (chemically induced dynamic *nuclear* polarization) or CIDEP (chemically induced dynamic *electron* polarization), to learn more about control mechanisms in fundamental chemical and biochemical photoreactions.

During these decades of independent meandering in the world of research, they occasionally encountered at laboratories and conferences around the globe, for example in Osaka, Sendai, Kyoto, Berlin, Padova, Kazan, or Novosibirsk. They discussed their ideas in science and society, and then separated again. But they always looked forward to their next encounter to again exchange new ideas and old experiences. (Takeji, the *Bunraku* puppet theater in Osaka was really a highlight to remember).

Evidently, there were stretches of time when our research interests overlapped dynamically in some respects. Hence, it was not too surprising when, suddenly, we realized that we have a common interest in the Möbius strip topology of expanded porphyrins. This very moment occurred in July 2011, during a symposium at the Max Planck Institute in Mülheim (Ruhr) that was organized by Wolfgang Lubitz in honor of the 75th birthday of Klaus Möbius. Seigo Yamauchi attended the symposium as an invited speaker. In his lecture, he reported—as a friendly guest gift, so to say—that in Sendai he and his co-workers have performed time-resolved EPR experiments on the photoexcited triplet state of a twisted expanded porphyrin, [28]hexaphyrin, with Möbius-strip topology. We realized that there are independent EPR studies going on simultaneously in
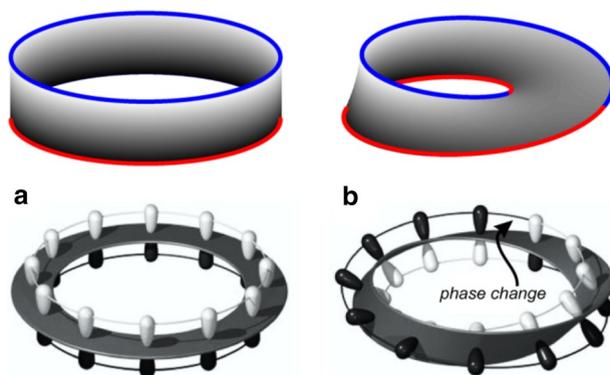

Fig. 2 Schematic representation of the topologies of π-conjugated electron systems of closed-loop molecules with a two-sided Hückel bracelet-like symmetry or a one-sided Möbius-strip-like symmetry. Gray and black lobes represent different phases by π (signs) of carbon $2p_z$ orbitals with respect to a molecular symmetry surface (dark gray). **a** In the planar Hückel topology, all the $2p_z$ orbitals of the loop have the same phase and are perpendicular to the two-dimensional, double-sided molecular surface (an orientable Hückel band looking like a bracelet). **b**. In the Möbius topology, a phase change by 180° (half-twist by π) between two neighboring $2p_z$ orbitals has occurred leading to a three-dimensional, one-sided molecular surface (a non-orientable band looking like a Möbius strip) to which locally the $2p_z$ orbitals remain perpendicular. (Adapted from [2])

Berlin, Sendai, Kobe, and Osaka on the existence of Möbius-type aromaticity of 180° twisted cyclic molecules with phase change of their $2p_z$ orbitals versus Hückel-type aromaticity of planar cyclic molecules.

Figure 2a represents the "standard" double-sided Hückel aromaticity, where all the 2pz orbitals of the planar carbon skeleton have the same phase with respect to the two-dimensional molecular plane as, for instance, in the benzene molecule. Figure 2b shows the half-twisted (180°) Möbius-aromatic molecule with a three-dimensional single-sided molecular surface, where two neighboring $2p_z$ orbitals have experienced a phase change by π (sign reversal of the $2p_z$ lobes).

2 Doublet and Triplet States of Expanded Porphyrins with Möbius Strip Topology

It took almost 40 years since Edgar Heilbronner (University of Basel) published a seminal paper [3] predicting Möbius aromaticity of sufficiently large twisted hydrocarbon molecules, until, in 2003, the first synthesis of an aromatic Möbius annulene was accomplished by Rainer Herges and co-workers at the University of Kiel [4]. And it took another four years before, in 2007, Lechosław Latos-Grażyński and co-workers at the University of Wroclaw [5] succeeded in synthesizing a new type of expanded free-base porphyrin compound that can adopt Möbius topology. Independently and at about the same time, Atsuhiro Osuka and his colleagues and co-workers at the Universities of Kyoto and Seoul succeeded in synthesizing and characterizing expanded porphyrin compounds with and without transition metals in their core that can adopt Möbius topology [6–9].

Excellent overviews of progress in the synthesis and physical characterization of expanded porphyrins with Möbius aromaticity are given by Latos-Grażyński and coworkers, e.g., [2, 5, 10, 11], as well as by Atsuhiro Osuka and co-workers, e.g., [8, 12, 13].

In 2007, Lechosław Latos-Grażyński and co-workers in Wroclaw synthesized free base di-p-benzi[28]hexaphyrin(1.1.1.1.1.1), an expanded porphyrin which can dynamically switch between Hückel and Möbius conjugation upon changes of solvent and temperature [2]. Shortly thereafter, in 2008, Atsuhiro Osuka and his co-workers from Kyoto, Seoul and Hyogo [8] accomplished the synthesis of an expanded porphyrin in which metalation triggered molecular twisting and Möbius aromaticity.

Porphyrins and their derivatives constitute an important class of biomolecules. They easily bind to metal cations to ligate the N-containing macrocycle. Metalated porphyrins perform various biological functions, for instance storing oxygen in hemoglobins or catalyzing photosynthetic electron transfer via chlorophylls. The specific free-base expanded porphyrin compound synthesized by the Wroclaw group can even switch between Hückel or Möbius aromaticity depending on the polarity and temperature of the solvent. Such a dynamic switching between Hückel and Möbius topologies with different electronic structures and properties by applying only small changes in external conditions (temperature, solvent, pH) or ring structure (meso-substituent, central metal) belongs to the most appealing applications

of [28]hexaphyrins. If properly controlled, molecular switches with unique optical and magnetic properties can thus be generated. In fact, hexaphyrin-type expanded porphyrins have already been approved as promising building blocks for switchable Hückel–Möbius complexes to be used as novel nonlinear optics devices. This is because Möbius and Hückel conformers exhibit large differences in their pronounced nonlinear optical properties [6, 14, 15].

In the following, we will put our emphasis on recent examples of electron magnetic resonance experiments on Möbius-type expanded porphyrins in their open-shell electronic states. We will begin with our EPR, ENDOR and DFT studies of doublet and triplet open-shell expanded porphyrin systems which have been performed in cooperation with Lechosław Latos-Grazynski and his coworkers in Wroclaw [16, 17].

Then, we will report on the work of the Japanese research team in Sendai, Osaka, Kobe and Kyoto dealing with the role of charge-transfer character in driving Möbius antiaromaticity in the excited triplet state of twisted [28]hexaphyrin [1].

2.1 Möbius–Hückel Topology Switching in Expanded Porphyrins: cw EPR, ENDOR, and DFT Studies of Doublet and Triplet Open-Shell Systems [16, 17]

Here, we report on our recent EPR, ENDOR, and DFT studies on open-shell states of Compound **1**, see Fig. 3. We give a summary of the ground-state radical cation doublet state (total electron spin $S = 1/2$) and the first excited triplet state ($S = 1$).
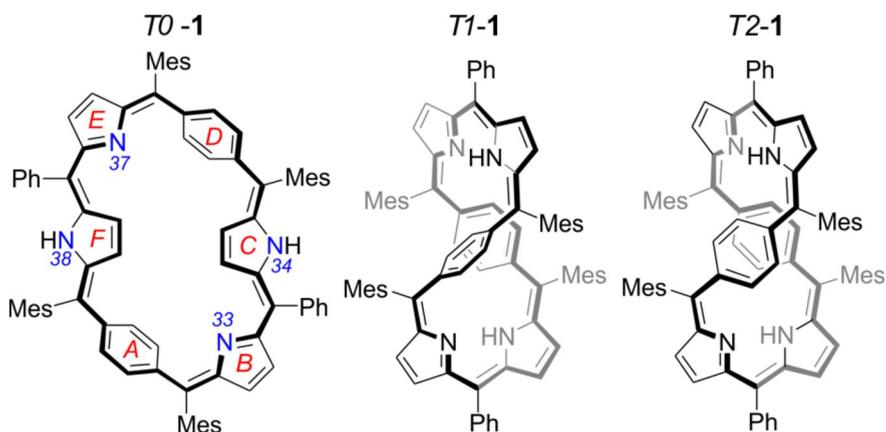
Compound **1**: Accessible conformations of free-base di-p-benzi[28]hexaphyrin(1.1.1.1.1.1)

Fig. 3 Compound **1**: free base di-p-benzi[28]hexaphyrin(1.1.1.1.1.1) and its spectroscopically accessible conformations of free-base [28]hexaphyrin **1** (Mes = mesityl, Ph = phenyl). The 28-π-electron conjugation pathway is shown in bold. A and D denote the two benzene rings in the conjugation pathway whose orientation relative to each other determine the topology of the expanded porphyrin molecule and thereby its type of aromaticity. The free-base expanded porphyrin **1** can adopt three conformers, abbreviated *T0*-**1** (Hückel), *T1*-**1** (Möbius) and *T2*-**1** (Hückel), with different π-surface topologies [5]. The cation radical of conformer *T0*-**1** was not observed in the present EPR study

The summary of the $S = 1/2$ system is largely based on a 2015 joint publication with the Latos-Grażyński group on radical cations of **1** [16]. The radical-cation study was the first one at all to deal with an open-shell π-system of an organic molecule with Möbius-strip topology. In the doublet state, the hyperfine interactions of the unpaired electron spin with specific magnetic nuclei in the molecule were used as sensitive probes for the electronic structure of the molecule and its symmetry and topological properties.

In 2016, this work has been extended to theoretical state-of-the art DFT MO studies on photo-excited triplet states of Compound **1** [17]. In the open-shell triplet state, besides hyperfine couplings, a change of the zero-field splitting interaction between the two unpaired electron spins is predicted to be a viable sensor for electronic-structure changes upon Möbius-to-Hückel topology switching. In the following, we will quote from this publication to summarize the results of our studies on cation radicals and excited triplets of Compound **1**.

Compound **1** was kindly provided by Lechosław Latos-Grażyński. It was synthesized in his group as described earlier [2, 10, 18]. The yield of the chemical synthesis is extremely low (ca. 5%) and renders isotope labeling by ^{15}N practically inaccessible. This would have been desirable as far as ENDOR spectroscopy is concerned to unambiguously assign the complex hyperfine structure to individual nuclei of the molecule [19].

Continuous wave (cw) EPR and ENDOR measurements at X-band microwave (mw) frequency (9.56 GHz) were performed with a computer-controlled ER 200D spectrometer (Bruker) upgraded with home-built ENDOR accessories, including a specialized TM_{110} mw cavity/ENDOR coil arrangement [20–22]. Thereby, the ENDOR detection sensitivity could be strongly enhanced which was a necessary requirement for this study because of the notoriously weak signals of the cation radicals of **1** (for details, see [16]). All experimental parameters, such as radical concentration, mw and rf power, as well as signal averaging methods, were varied to optimize the cw ENDOR signal-to-noise ratio. Liquid-solution EPR and ENDOR spectra of the radical cations of **1** were recorded in dichloromethane (CH_2Cl_2) over a temperature range between 190 and 330 K. This wide temperature range was chosen to get the largest possible Boltzmann population differences between the two topological conformers, i.e., the Möbius form *T1*-**1** (dominant at low temperatures) and the Hückel form *T2*-**1** (dominant at high temperatures). The switching behavior between Hückel and Möbius aromaticity depends, among other parameters, on the polarity of the surrounding solvent [2]. CH_2Cl_2 is a favorable solvent because of its large polarity, $\varepsilon(CH_2Cl_2) = 9.1$.

The radical cations of Compound **1** in CH_2Cl_2 were generated coulometrically under controlled potential at room temperature. The coulometry was performed under high-vacuum conditions in a home-built electrolysis cell using $TBAPF_6$ (tetrabutylammonium hexafluorophosphate) as supporting electrolyte, see [16]. Cyclic voltammetry of **1** was measured before performing coulometry to assure reversibility of the one-electron oxidation reaction. An EPR pyrex sample tube (3 mm od; 2 mm id) was directly fused to the coulometry cell to allow for simultaneous voltammetry and EPR measurements.

The concept of Möbius topology as a stabilizing factor for aromaticity in expanded organic molecules has gained strong momentum in recent years from applications of quantum chemistry theory on such systems employing large-scale calculations up to the level of modern DFT [2, 23–27]. It treats all valence electrons on an equal footing, i.e., does not differentiate *a priori* between σ- and π-electrons, as basic HMO theory does which, in fact, had been employed in Heilbronner's seminal work from 1964 [3].

Computational chemistry at the DFT level stood the test of aiding molecular design of viable Möbius-to-Hückel switches, since it can provide a full description of transitions between Möbius and Hückel aromaticity. In that respect, state-of-the-art quantum-chemical computations can suggest "virtual" experiments for deciding on the aptitude of a specific molecule for topology switching (see below).

Until very recently (2015), only ground-state *closed-shell* Möbius π-electron systems have been studied both theoretically and experimentally. But that has changed. In the preceding publication [16], the EPR and ENDOR studies on doublet-state cation radicals of a free-base [28]hexaphyrin with Möbius-strip topology were reported. It turned out that the open-shell cation radicals still exhibit a Hückel–Möbius aromaticity switch, similar to what the closed-shell neutral precursors do. The switching ability is the result of solvent- and temperature-controlled changes in the electronic and molecular topology. For the doublet-state radical cations, the adequate spectroscopic tool for characterizing such switchable topologies in detail was, not unexpectedly, EPR together with its multiple-resonance extension ENDOR and state-of-the art DFT MO calculations.

First, we will report our preceding EPR and ENDOR results on the *open-shell* cation radicals of Compound **1**. After that, we will report the results of our theoretical DFT studies on the electronic structure of the photoexcited *open-shell* triplet state of Compound **1**. The emphasis lies on the comparison of the hyperfine and zero-field splitting parameters of the Hückel and Möbius conformers in their doublet and triplet states.

The interesting question is whether both the *local* properties of the electronic structure, as probed by spin densities and hyperfine couplings, and the *global* properties, as probed by the zero-field splitting, do reflect the molecular topology of the Möbius and Hückel conformers in their respective open-shell states. The quantum-chemical MO calculations of magnetic spin-interaction parameters like hyperfine couplings, g- and zero-field tensor components as well as total energies of the large porphyrin molecules were performed on the ORCA level of DFT approximation [28], see below.

2.1.1 Cation Radicals of Compound 1

The motivation for extending EPR to ENDOR is to improve the spectral resolution of electron-nuclear hyperfine interactions for large molecular complexes. With each set of inequivalent nuclei in a low-symmetry molecule the number of EPR lines increases in a *multiplicative* way. Evidently, this results in inhomogeneously broadened EPR spectra in which individual hyperfine lines can no longer be resolved. In ENDOR, on the other hand, for the same low-symmetry molecule the number

of lines increases only in an *additive* way, like in NMR. In ENDOR, the sample is irradiated simultaneously by two electromagnetic fields, a microwave (mw) field (to drive EPR transitions) and a radio-frequency (rf) field (to drive NMR transitions). Under appropriate saturation conditions [19], ENDOR signals are detected by monitoring the changes of EPR line amplitudes when sweeping the rf field through the NMR frequencies of specific nuclei of interest. Thus, ENDOR is considered a very sensitive variant of NMR of paramagnetic systems, the unpaired electron serving as a sensitivity-boosting detector. When nuclei with different magnetic moments are involved, e.g., ^{1}H and ^{14}N nuclei, their ENDOR lines appear in different rf frequency ranges, which are determined by the Larmor frequencies of these nuclei.

The observed line splittings in the ENDOR spectra of the radical cations of Compound **1** in liquid solutions are caused by the isotropic hyperfine interactions (scalar "Fermi contact" interaction) of the delocalized unpaired electron with the various nuclei n of the molecular skeleton. The value of the scalar hyperfine coupling constant (hfc), a_n, is proportional to the squared amplitude of the electronic wavefunction at the associated nucleus n [29], e.g., $n = {}^{14}$N or ^{1}H. It can be calculated by a widely accepted quantum chemical DFT algorithm [30, 31] which is an essential component of the versatile software package ORCA (Version 3.0) developed by Frank Neese and co-workers [28]. It allows to choose from a large number of different functionals and atomic basis-sets; this is an essential part of the program settings for performing a meaningful DFT calculation.

For a set of a_n values, in first-order high-field approximation, the ENDOR line positions are given, in linear frequency units, by [19]

$$\nu_{\text{ENDOR}} = \left| \nu_n \pm a_n/2 \right|, \tag{1}$$

i.e., two ENDOR lines, symmetrically placed around the free nuclear Larmor frequency $\left| \nu_n \right|$ or $|a_n/2|$, whichever is larger, are expected for each set of symmetry-equivalent nuclei n with an hfc a_n.

The hyperfine interactions with magnetic nuclei ($I \neq 0$) in the molecule can be used as sensitive local probe for the electronic structure and symmetry properties of the molecular state [19]. Such symmetry aspects are particularly pronounced in ENDOR since they often allow one to identify the topology of the observed species by merely counting the number of lines in the ENDOR spectrum. This number is determined by the symmetry of the molecule. Thus, for a planar Hückel structure with its higher symmetry, we expect fewer ENDOR lines than for a twisted Möbius structure with its lower symmetry.

The main objective of the present study is to examine whether a Hückel–Möbius aromaticity switch in [28]hexaphyrin **1** exists not only in its closed-shell singlet ground state ($S=0$) [32] but also in its open-shell doublet and triplet states. The experimental ENDOR results clearly show that at high temperatures the Hückel-conjugated conformer with its higher symmetry dominates, whereas at low temperatures the Möbius-conformer with its lower symmetry dominates.

The details of the EPR and ENDOR spectra analysis and theoretical conclusions have been presented in [17] and shall not be repeated here. In the following, we give

only a summary of the results and discussion, but for the full arguments we refer the reader to our original publication.

For the quantum chemical MO calculations of the electronic structures of the conformers of Compound **1**, the nuclear coordinates were adopted from the publication of Stępień and co-workers [5]. Their data listings contain the Cartesian coordinates for the DFT-optimized structures of both the Möbius (*T1*-**1**) and Hückel (*T2*-**1**) molecules in their neutral singlet ground states. These structures were obtained by minimum-energy geometry optimization with the functional B3LYP [30, 31] and basis set 6-31G** [33] on predetermined single-crystal X-ray structures. They comprise altogether 158 atoms (82 C, 72 H, 4 N). All calculations were performed on the HERMES supercomputing cluster at the Max Planck Institute for Chemical Energy Conversion in Mülheim (Ruhr), with 65 nodes, each having 12 cores and 48 GB RAM.

The EPR spectra of the cation radicals of *T1*-**1** and *T2*-**1** show inhomogeneously broadened Gaussian lines typical for an unresolved hyperfine structure of the large number of magnetically inequivalent ^{1}H and ^{14}N nuclei in the molecule. Accordingly, the EPR spectra lost most of their structural information and are indistinguishable for the *T1*-**1** and *T2*-**1** conformations. Hence, we used ENDOR with its inherently higher resolving power for small hyperfine couplings. But, even the ^{1}H-ENDOR spectra contain a large number of lines (> 70), which overlap in the narrow frequency range between 12 and 17 MHz and are exceedingly difficult to disentangle. For this reason, we limit the present summary to the discussion of the ^{14}N spectra of the cation radicals of Compound **1** for which a maximum of 8 lines is expected for symmetry reasons. The ^{14}N ENDOR results and most of the ^{1}H-ENDOR results are discussed in detail in [16] where the complete experimental and theoretical data sets are given.

According to Eq. (1), the ^{14}N-ENDOR spectra are expected to contain at most 8 separated lines from the four nitrogens over the frequency range 0–4.5 MHz. To avoid a mixture of populations of both topologies, we focused on the "low" temperature (≤ 230 K) and "high" temperature (≥ 330 K) regions, where the corresponding conformers should appear in more or less pure concentrations [2].

Ultimately, isotropic ^{14}N and ^{1}H hfc's were derived from the ENDOR spectra for the two topologies, Möbius and Hückel. Theoretical ORCA-DFT ^{14}N values for a_n served as guideline for checking consistency. The results were supported by corresponding spectra simulations.

The pattern of all the theoretical ^{14}N and ^{1}H isotropic hfc's in the radical cations of *T1*-**1** and *T2*-**1**, as determined by the ORCA-DFT calculations, are visualized in Fig. 4.

As an essential feature, the theoretical results reflect the increase of molecular symmetry when switching from the Möbius to the Hückel structure, in that both nitrogen and proton isotropic hyperfine couplings appear practically pairwise equivalent in *T2*-**1**. As potential candidates for structural changes, we considered the N–H bond lengths as well as various C–C and C–N bond lengths in the macrocycle. Changes in the attached mesityl and phenyl rings (see Fig. 3) turned out to be negligibly small (< 0.001 Å). The largest changes (≈ 0.03 Å) occur in the C–C bonds between the macrocycle and the benzene ring having its plane positioned vertically
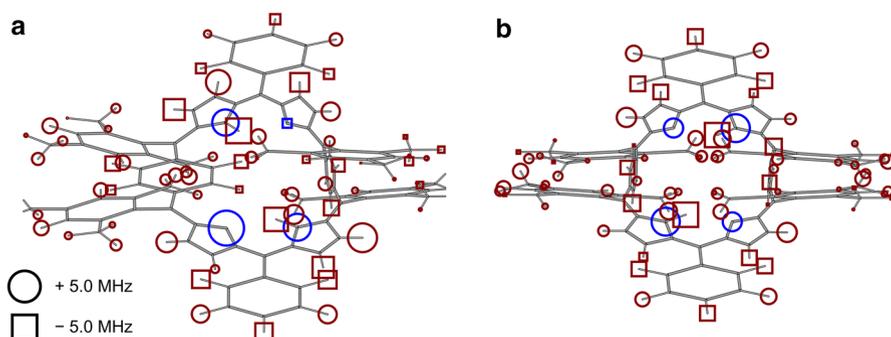
Fig. 4 Open-shell cation radical state. Theoretical hyperfine couplings (ORCA-DFT) in *T1*-**1** (**a**, Möbius) and *T2*-**1** (**b**, Hückel). Maroon and blue circles or squares refer to protons and nitrogens, respectively. The hyperfine couplings are proportional to the areas of the respective squares (hfc $a < 0$) and circles ($a > 0$). For details, see [17]

to the mean molecular plane. Despite the smallness of all these geometrical changes, the effects on particular hfc's are quite significant. This demonstrates the strong dependence of the various *local* isotropic hyperfine interactions on the large-scale (*global*) features of the molecular orbital (HOMO) carrying the unpaired electron. For details, see [16].

2.1.2 Excited Triplet State of Compound 1

As an important extension of the previous research on the cation radicals, we considered additional DFT and EPR studies on the relationship between Hückel/Möbius aromaticity and topological properties of [28]hexaphyrins in their first excited triplet electronic configurations. First theoretical results were reported in 2016 [17]. Our theoretical studies were again based on ORCA-DFT calculations. Decisive information on the extent of triplet-state delocalization is anticipated from the *global* zero-field splitting (ZFS) interaction between the two unpaired electrons in the triplet-state molecule [29]. Zero-field splitting information is complementary to the information obtained from the *local* isotropic hyperfine interactions with the various magnetic nuclei in the molecule. Thus, we expect the ZFS parameters of the EPR spectra to be sensitive indicators of prevailing *T1-1* or *T2-1* triplet species.

Quantitatively, the ZFS interaction can be described by an effective spin Hamiltonian of the form

$$\hat{H}_{\mathrm{D}} = \hat{S} \cdot \mathbf{D} \cdot \hat{S}, \tag{2}$$

where **D** is a symmetric tensor called the ZFS tensor. Its components are given by the quantum mechanical average over the electronic triplet wave function:

$$D_{ij} = \tfrac{1}{2} g^2 \mu_{\mathrm{B}}^2 \cdot \left\langle \left(r_{12}^2 \delta_{ij} - 3 i_{12} j_{12} \right) / r_{12}^5 \right\rangle, \tag{3}$$

with $i, j = x, y, z$ [29]. Here, the subscripts 1, 2 refer to the two unpaired spins. Thus, the D_{ij} values are determined by the spatial distribution of the two unpaired spins and their relative orientation.

In terms of the principal axes system that diagonalizes the ZFS tensor, the Hamiltonian (Eq. 3) becomes

$$\hat{H}_D = -X\hat{S}_x^2 - Y\hat{S}_y^2 - Z\hat{S}_z^2, \tag{4}$$

where $-X$, $-Y$ and $-Z$ are the principal values of the ZFS tensor. Since the ZFS tensor is traceless ($X + Y + Z = 0$), $\hat{H}_D$ in Eq. 4 can be rewritten in terms of just two independent parameters, D and E, the "fine-structure" or "zero-field-splitting" parameters:

$$\hat{H}_D = D \cdot \left(\hat{S}_z^2 - 1/3\,\hat{S}^2\right) + E \cdot \left(\hat{S}_x^2 - \hat{S}_y^2\right), \tag{5}$$

with

$$\begin{aligned} D &= 3/4 g^2 \mu_B^2 \cdot \left\langle \left(r_{12}^2 - 3z_{12}^2\right)/r_{12}^5 \right\rangle \\ E &= 3/4 g^2 \mu_B^2 \cdot \left\langle \left(y_{12}^2 - x_{12}^2\right)/r_{12}^5 \right\rangle. \end{aligned} \tag{6}$$

The same ORCA software package as used for calculating the hyperfine couplings also provides an option for a spin-unrestricted DFT calculation of the ZFS tensor of excited triplet states. In detailed calibration work [34] it was found that the spin-unrestricted DFT methods behave "somewhat erratically" and that much more accurate values of D and E were obtained from open-shell *spin-restricted* DFT employing *spin-unrestricted* "natural orbitals", abbreviated UNO [34].

The corresponding results for the triplet-state conformers of *T1-1* (Möbius) and *T2-1* (Hückel) are (in wave number and frequency units, 10^{-4} cm^{-1} ↔ 3 MHz):

$T1 - \mathbf{1}:$ $D = +181 \times 10^{-4}$ cm^{-1}, $E = +53 \times 10^{-4}$ cm^{-1} ($D = +543$ MHz, $E = +159$ MHz)

$T2 - \mathbf{1}:$ $D = -133 \times 10^{-4}$ cm^{-1}, $E = +16 \times 10^{-4}$ cm^{-1} ($D = -399$ MHz, $E = +48$ MHz).
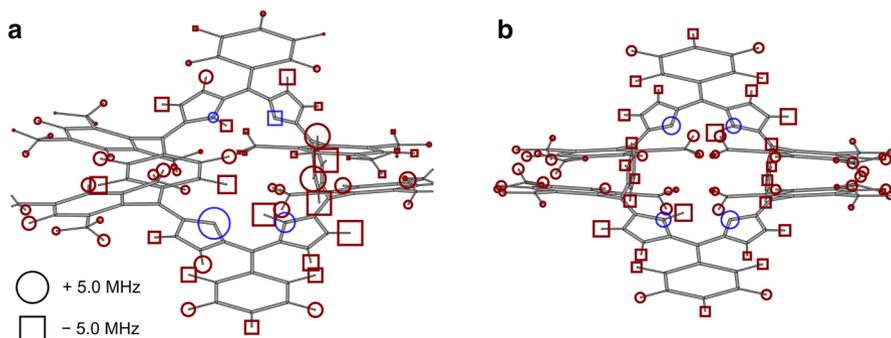

Fig. 5 Open-shell excited triplet state. Theoretical hyperfine-couplings (ORCA-DFT) in *T1-1* (**a**, Möbius) and *T2-1* (**b**, Hückel). Maroon and blue circles or squares refer to protons and to nitrogens, respectively. The hyperfine couplings are proportional to the areas of the respective squares (hfc $a < 0$) and circles ($a > 0$). For details, see [17]

Of particular interest is the change of sign of the ZFS parameter D between the two conformers, *T1-1* (Möbius) and *T2-1* (Hückel). This result can be rationalized by inspection of the different spin distributions in the two conformers (see Fig. 5).

The *D* value for a cigar-shaped (prolate spheroid) spin distribution is negative while, with the same average inter-electron distance, the *D* value for disk-shaped (oblate spheroid) spin distribution is positive. Such sign changes of D in extended π-systems have often been observed in EPR spectroscopy of biradicals or triplet states [35, 36] and interpreted as oblate-to-prolate spin-density transition, with an accompanying reorientation of the ZFS tensor. Similar to what was observed for the cation radicals of *T1-1* (Möbius) and *T2-1* (Hückel), the theoretical triplet state results also reflect the increase of symmetry when switching from Möbius to Hückel topology: the appearance of equivalent pairs of hyperfine couplings. The spin densities in *T2-1* are predominantly located on the spatially well-separated rings A and D ($r_{AD} \approx 5$ Å), see Compound **1** in Fig. 3, while the spin densities in *T1-1* are more strongly localized on only one of these rings (which is rotated perpendicularly out of the plane of the remaining macrocycle).

This is also found in biradicals with well-separated unpaired spins (cigar-like spin distribution, $D < 0$). In contrast, a positive sign of D is theoretically predicted by our DFT MO calculations for *T1-1* (Möbius), as is found, for example, for the triplet state of naphthalene with its unpaired spins located in a spatially confined region (disk-like spin distribution) [29]. Thus, the DFT computational results provide evidence for a different π-electron delocalization in the first excited triplet state of the [28]hexaphyrin conformers, accompanied by a reorientation of their ZFS tensor with respect to the molecular frame from prolate to elongate spheroid when switching Compound **1** from Hückel to Möbius topology.

From the results of the DFT MO calculations we conclude that the ZFS tensor parameter D is indeed a sensitive probe for identifying topology switching in [28] hexaphyrins in their excited triplet state. The relationship between the aromaticity/antiaromaticity and the electronic structures of twisted molecular complexes is highly informative for a better understanding of the fundamental chemical reactivity of Möbius aromatic molecules in their ground and excited states. This is vital for future theory-aided designs of functional organic molecules. For example, the ability of novel expanded porphyrins for switching between Hückel and Möbius topologies opens up the possibility of developing new types of storage devices in computer technology.

2.2 Charge-Transfer Character Drives Möbius Antiaromaticity in the Excited Triplet State of Twisted [28]hexaphyrin: A Time-Resolved EPR (TREPR) Study [1]

In recent years, Möbius-type aromatic macrocyclic molecules have been extensively synthesized. Their aromatic or antiaromatic characters have been confirmed experimentally in singlet S_0 states, but their excited electronic properties, particularly in T_1 states, have been scarcely studied so far.

It is gratifying to note that recently (2018), in a concerted effort, 12 Japanese scientists from Universities in Kobe (Yasuhiro Kobori and coworkers), Kyoto (Atsuhiro Osuka and coworkers), Osaka (Takeji Takui and coworkers), and Sendai (Seigo Yamauchi, who sadly passed away in September 2012), published the first experimental EPR study on the photoexcited triplet state of the twisted Möbius expanded porphyrin [28]hexaphyrin and compared it with the planar Hückel expanded porphyrin [26]hexaphyrin [1]. In their study, they examined the T_1 states of meso hexakis(pentafluorophenyl) [26]hexaphyrin(1.1.1.1.1.1) ([**26**]**Hex**) and meso-hexakis-(pentafluorophenyl) [28]hexaphyrin (1.1.1.1.1.1) ([**28**]**Hex**). They described in detail the electronic character of T_1 of Möbius [28]**Hex**, employing TREPR (time-resolved EPR) in conjunction with magneto-photoselection measurements at 77 K. They performed advanced DFT MO calculations for interpreting the experimentally determined zero-field tensor orientation in T_1.

For photoexcitation of [28]**Hex**, they used either unpolarized light pulses or laser light pulses linearly polarized parallel or perpendicular to the direction of the external magnetic field B_0. The line shape of the delay-time-dependent TREPR spectra of [28]**Hex** changes depending on whether irradiating with depolarized light, or with the light (**L**) parallel to $\mathbf{B_0}$ ($\mathbf{L} \parallel \mathbf{B_0}$), or with the light perpendicular to $\mathbf{B_0}$ ($\mathbf{L} \perp \mathbf{B_0}$). This indicates that the electron spin polarization changes with the polarization directions of the laser light (**L**). It demonstrates that the broadening of the TREPR spectra originates in peak splittings by anisotropic spin–spin dipolar coupling in the T_1 state. The rationale behind such a statement is that the tensor orientation of the zero-field interaction is defined with respect to the optical transition dipole moment in the molecule. Identification of the ZFS tensor orientation could thus be achieved by analyzing the TREPR spectra. It enabled the authors to characterize the electronic structures of the T_1 states in unprecedented detail. The results were interpreted by means of DFT MO model calculations of the zero-field parameters D and E. They involved both local $\pi\pi^*$ excitations (LE) and intramolecular charge-transfer (CT) excitations between orthogonal unpaired orbitals. In the DFT-MO model calculations, the pentafluorophenyl groups of planar [26]**Hex** and twisted [28]**Hex** were replaced by hydrogen atoms.

As main results, the authors showed that the unpaired electronic orbitals in the T_1 state are not uniformly delocalized over the macrocycle of the twisted Möbius [28]hexaphyrin at cryogenic temperature. Rather, their analysis of the EPR parameters revealed that the T_1 state possesses substantial intramolecular charge-transfer character together with local excitation character residing predominantly at one side in the Möbius ring structure. As a consequence, the unpaired electrons in twisted Möbius [28]hexaphyrin are separated from each other, resulting in a *negative* zero-field splitting parameter ($D < 0$). In contrast, because [26]hexaphyrin is a planar Hückel aromatic molecule, the D parameter of the triplet state T_1 is expected to be $D > 0$. The authors conclude that it is the charge-transfer character of the triplet wave function that drives Möbius (anti)aromaticity in the first excited triplet state of twisted [28]hexaphyrin. The contributions of the CT and LE characters were estimated to be CT:LE $\approx$ 1:5.

For the ZFS Parameters D and E, the authors present as final results [1]:

$$[\mathbf{28}]\mathbf{Hex}(\text{Möbius}) \quad D = -532\,(\pm 3)\,\text{MHz}, E = -18.2\,(\pm 2)\,\text{MHz}$$
$$[\mathbf{26}]\mathbf{Hex}(\text{Hückel}) \quad D = +813\,(\pm 3)\,\text{MHz}, E = -205\,(\pm 3)\,\text{MHz}.$$

It is thus concluded that for [28]**Hex** (Möbius) the sign of D is *negative* with the principal Z-axis directing along the molecular long axis. This also specifies that the spin density distribution is not fully delocalized in the [28]**Hex** macrocycle in the T_1 state, in line to what is predicted by the DFT MO calculations. From the line shape analysis to fit the TREPR spectra, the authors obtained the principal axes directions of the ZFS interaction of [28]**Hex** for the negative D using the polar ($\delta = 60°$) and azimuthal ($\gamma = 40°$) angles with respect to the transition dipole moment **M** of [28]**Hex**, as determined by magnetophotoselection. It is noted that the computed principal axes orientation of the spin–spin dipolar interaction is consistent with the experimentally determined orientation.

These TREPR/magnetophotoselection and DFT MO results are in contrast to our earlier purely theoretical prediction of a *positive D* parameter ($D > 0$) of triplet state *T1*-**1** (Möbius topology) [17], as presented above. The sign discrepancy is most probably caused by the neglect of significant charge-transfer contributions in the DFT MO calculations of triplet state *T1*-**1** (Möbius topology) in our 2016 study [17].

Aromaticity is one of the most common concepts in chemistry. Chemical properties and reactivities of π-conjugated macrocyclic molecules depend on their aromatic or antiaromatic character. The 2018 paper by Fumitoshi Ema et al. [1] received wide attention in the chemistry community, see for instance *ScienceDaily, 14 May 2018* [37]. From this source, we quote Yasuhiro Kobori from Kobe University, who commented: "The special electronic properties of this highly active excited state could be applied in electronic functional materials, such as organic solar cells and electric conductors, and could potentially contribute to the solution of energy and environmental issues."

3 Outlook to Medical Applications: The Cyclotide Mini-proteins with Trefoil-Knot and Möbius-Strip Topologies

In this section, we want to give special recognition to David Craik, professor of Molecular Bioscience at the University of Queensland, Australia. He was among the first scientists to discover the cyclotide family of globular circular proteins, and pioneered their biomolecular synthesis. Through his discoveries he has opened new horizons for designing enzymes and pharmaceutical drugs of exceptionally high chemical and medical stability [38].

Cyclotides represent a new class of natural plant proteins involved in plant defense and protection. They are circular mini-proteins, i.e., small proteins of various folding types, usually less than100 amino acids long. They are distinguished by exceptional chemical and biological stability and, hence, are considered ideal pharmaceutical "template" molecules for protein drug design. This chemical and biological stability is rare among proteins and should allow them to cross the blood–brain barrier when applied to humans. This barrier is a highly selective semipermeable border that separates the circulating blood from the brain and extracellular fluid in

the central nervous system. Hence, it is anticipated that the cyclotide drugs have sufficient stability in the human gut to keep them active after oral administration to clients, a property almost unique in medical peptide drug therapy.

The tertiary structure of Cyclotides is characterized by a circular cystine-knot motif (the "cys-knot"), comprising an amino-acid chain. It is stabilized by three conserved disulfide bonds in a knotted arrangement, and a head-to-tail cyclized peptide backbone. Möbius topology combined with a characteristic knot motif is a distinctive feature of certain members of the unique family of cyclic and knotted proteins.

Recent advances in bioengineering have made it clear that the properties of a new (bio)chemical material are determined not only by its chemical composition but also by geometrical and topological factors [39]. In light of this realization, and the recent breakthrough advances in fabricating objects with molecular precision, research to exploit the one-sided Möbius strip topology for scientific and medical applications is becoming increasingly intense.

There is an intrinsic connection between knot theory and topology theory of Möbius strips. The number of knots is linked to the boundary (edge) of Möbius strips with their number of half-twists. Möbius strips exhibit only a single edge and a single side (surface). With one half-twist (180°), the edge of the Möbius strip forms the *unknot*, 0_1, and with three half-twists, the edge of that Möbius strip forms the *trefoil-knot*, 3_1.

The situation of topology considerations for synthetic chemists and bioengineers has changed with the discovery of the cyclotides, those cyclic disulfide-rich miniproteins that can be isolated from certain plants. Their tertiary structure is typically maintained by disulphide bridges. Their extraordinary chemical and biological stability can be rationalized in terms of knot theory—and no longer solely through the Möbius-strip topology of the band-shaped molecular fragments and the orientation of their π orbitals, as was discussed in Sect. 2 for the case of expanded porphyrins.

Normally, when linear proteins are orally administered as tablets or teas, they have a major problem, the body's digesting system would consider them as food that needs to be disintegrated into the constituents for effective metabolism. Our bodies have evolved to break polypeptides down quickly to form amino acids. In fact, this limits the clinical use of peptide pharmaceuticals in general, specifically when oral application is needed. Hence, while the major drawbacks of the present-day protein drugs in medical treatment are the toxins' inability to cross the blood–brain barrier because of their low chemical and biological stability, the situation is different in the case of the cyclotides. With them the "impossible" has become possible.

Actually, it is not surprising that Nature is ahead of us in inventing the "impossible". Some complex biomolecules and biopolymers have been isolated from natural systems which wind themselves into a knot topology. A prominent example is the cyclotide *Kalata B1* that adopts both Möbius-strip and trefoil-knot topologies, as is shown in Fig. 6.

We will restrict ourselves to the topic of molecular topology of the standard Möbius strip (one half-twist) and the triply twisted Möbius strip (three half-twists) and the respective *unknot* and *trefoil* knots of their boundaries, taking the cyclotides as example.
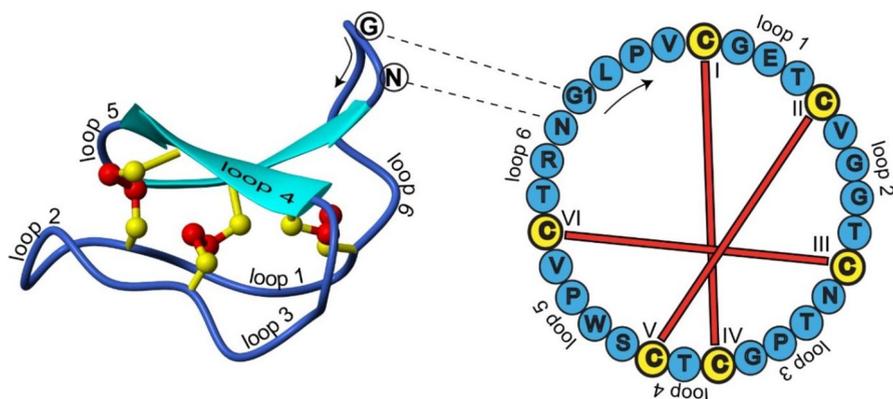
Fig. 6 Three-dimensional structure and sequence of the prototypic cyclotide *Kalata B1*. Cyclotides have a well-defined 3D structure as a result of their three interlocking disulfide bonds and cyclic peptide backbone. The cyclotide is represented by a "backbone wheel", and their backbone sequence is indicated by single letter amino acid representation. Six of the amino-acid residues cysteine form a trefoil-knot topology (the "cys-knot") that greatly enhances chemical and medical stability. In the case of *Kalata B1*, the indicated glycine (G) and asparagine (N) amino acids are the terminal residues that are linked in a peptide bond to cyclize the peptide, i.e., give it a circular backbone [40]

Although no cyclotides have reached human clinical trials yet, the results obtained with several bioactive cyclotides in animal models hint that this may occur in the near future. A leading figure in this field is Christian Gruber, biochemist at the Medical University of Vienna and former doctoral student of David Craik. He is currently investigating the influence of cyclotide natural products on autoimmune diseases. He was awarded the Science Prize of the European Federation for Medicinal Chemistry for his research achievements. In 2016, Gruber and coworkers had presented data on the influence of cyclotides on mice suffering from a disease similar to multiple sclerosis in humans. The cyclic peptide stopped the progression of the disease in sick animals. If the mice were given the active substance at an earlier stage, the symptoms did not break out at all. No side effects were observed. Several pharmaceutical companies are now interested in developing drugs from the cyclotides.

In the following we give a short excerpt from the highly acclaimed 2016 professional article of the Christian Gruber research group and their coworkers in PNAS [41].

Multiple sclerosis (MS) is the most common autoimmune disease affecting the central nervous system. It is characterized by autoreactive T-cells that induce neuronal degradation. Treatment options are still limited and, generally, MS medications have to be performed by injection, using a needle and a syringe, but are only modestly effective. In particular, if the drugs have to be administered via an injection into the spinal canal, it is particularly questionable, because it is dangerous and suffers from the toxin's inability to cross the blood–brain barrier.

Consequently, there is a demand for novel, especially orally, i.e., patient-friendly, active therapeutics. Recently, the circular plant peptide *Kalata B1* was shown to silence

T-cell proliferation in vitro. Owing to this promising effect, the Gruber team aimed to determine in vivo activity of the cyclotide mutant [T20K]kalata B1 (abbreviated T20K). The abbreviation [T20K]kalata B1 means a point-mutation substitution of Thr-20 with Lys (Threonine, Lysine) at position 20 of loop 4 in *Kalata B1* [42].

The authors used the MS mouse model *experimental autoimmune encephalo-myelitis* (EAE) which is an established animal model of brain inflammation caus-ing disease of the central nervous system including multiple sclerosis. Treatment of mice with the cyclotide [T20K]kalata B1 resulted in a significant delay and dimin-ished symptoms of EAE by oral administration. Cyclotide application substantially impeded disease progression and did not exhibit adverse effects.

Based on these data, the T20K cyclotide is on its way to clinical applications, using chemical modification strategies to generate highly active immunosuppressive cyclotides [42].

The present state of the Gruber model studies demonstrates that such strategy represents an innovative approach to design new drugs with immunomodulatory capacities. There is a justifiable hope that the major drawbacks of the present MS treatment, such as the toxins' inability to cross the blood–brain barrier, their low stability, and lack of oral activity, can be overcome in the near future by applying tailor-made cyclotides. The authors point out that the significance of such studies on multiple sclerosis can hardly be overestimated. MS imposes substantial social and economic burdens on patients, their families, and society. Their work provides a proof of principle that nature derived cyclic peptides serve as oral active therapeu-tics, utilizing their intrinsic bioactivity and stable three-dimensional structure due to topological constraints imposed by trefoil-knot and Möbius-strip formation. As a result, their physical and biochemical qualities are "topologically protected".

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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