Indeed, there is some disagreement about the efficacy of a ketogenic diet in previous studies, and heterogeneity may be one reason. Sada *et al.* address this issue by showing that LDH inhibition hyperpolarizes diverse types of principal cells (neurons that extend axons to the next brain area in a functional pathway to mediate seizures), including those in the substantia nigra and in the hippocampus. Thus, LDH inhibition is effective across different brain areas. Interestingly, blocking LDH failed to affect GABAergic neurons, showing remarkable specificity for principal cells that generate seizures and not the interneurons that inhibit the principal cells.

Reducing pyruvate in cultured neurons results in dehydrogenation of lactate, which was protective against hyperactivity. However, increasing the exogenous pyruvate concentration was beneficial in previous experiments using different endpoints (*11*, *12*). This discrepancy suggests that LDH inhibition will potentially be important in many contexts, but it is likely that the effect of blocking this enzyme will depend on how it is altered and where (e.g., neurons versus astrocytes). Fortuitously, it is possible to manipulate neurons selectively because LDH1 (one of the five forms of LDH) is primarily neuronal (*9*).

In the short time that epileptic rodents were treated with stiripentol in the Sada *et al.* study, there was remarkable efficacy of LDH inhibition. Would it have continued? Metabolism is remarkably resilient, and compensatory changes are common. Would there be side effects, given the widespread and complex nature of metabolism? Sada *et al.* offer much food for thought as well as fuel for the fire between those who focus on the neurons to control epilepsy, and those who champion the astrocytes. ■


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VIBRATIONAL DYNAMICS

# Taming $CH_5^+$, the "enfant terrible" of chemical structures

## Ion-counting spectroscopy reveals the low-energy states of a molecule with highly dynamic bonds

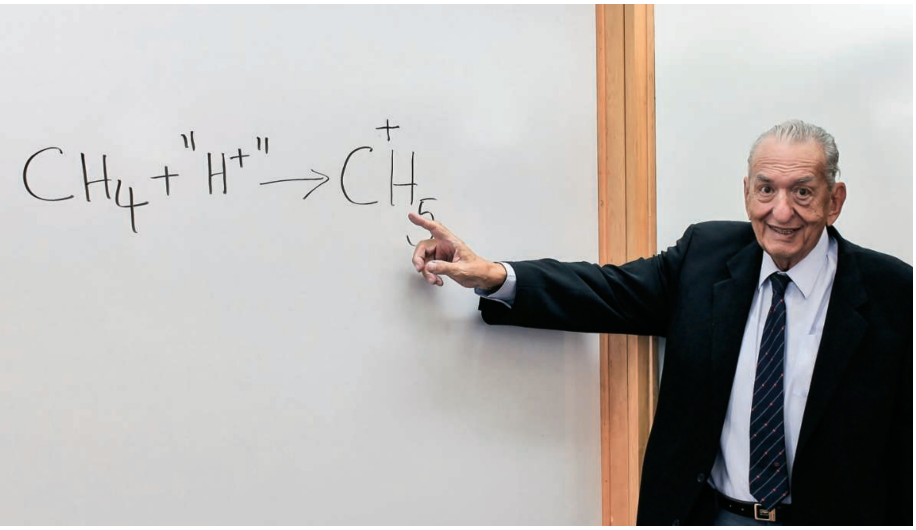
*By* **Takeshi Oka**

Protonated methane, $CH_5^+$, is a quantum dynamical system that challenges our understanding of chemical bonding and structure. The bonding does not lead to a trigonal bipyramid. Instead, the five protons swarm around the central carbon, and this gives rise to an incredibly complex vibration-rotation-tunneling infrared spectrum (*1*), making it an "enfant terrible" for spectroscopists. Ab initio theory has found that "there is essentially no barrier to hydrogen scrambling" (*2*) and "the very concept of molecular structure becomes problematic for this molecule" (*3*). For its parent molecule, $CH_4$, each rotational level corresponds to one quantum state, but for $CH_5^+$ it corresponds to 2 × 5! = 240 states. However, on page 1346 of this issue, Asvany *et al.* (*4*) report combination differences (CoDiffs) of the low-energy levels of $CH_5^+$, a first step at "taming" its spectrum.

For me, these results are exciting because I have been on the trail of this carbocation for decades. After I reported the infrared spectrum of $H_3^+$ in 1980 (*5*), I received a letter from George Olah, in which he asked, "I wonder whether a similar technique would enable to observe the infrared spectrum of $CH_5^+$. It is of great interest to organic chemists…." I did not know Olah or $CH_5^+$, the pivotal reaction intermediate in Olah's superacid chemistry that would lead to his 1994 Nobel Prize (see the figure) (*6*). I was simply delighted that my work of astrophysical interest was also important for organic chemists.

> **"…the very concept of molecular structure becomes problematic for this molecule…"**

The "similar technique" became a general method with the invention by Saykally of velocity modulation detection (*7*), which allowed the study of protonated ions, such as $NH_4^+$ and $H_3O^+$ (*8*). However, for plasmas containing $CH_4$, we obtained extremely complicated spectra with over 10,000 lines.




**Superacids and interstellar chemistry.** The discovery of the infrared spectrum of $H_3^+$, a strong proton donor (acid) central in interstellar chemistry, prompted the query from George Olah to search for $CH_5^+$. This molecule is pivotal in the superacid chemistry pioneered by Olah. The molecular bonding is fluxional and its spectrum is extremely complicated, but the high-resolution ion-counting spectroscopy at 4 K and 10 K observed by Asvany *et al.* identify crucial CoDiffs of low-energy states.

PHOTO: THOMAS MATHEW/LOKER HYDROCARBON RESEARCH INSTITUTE AND DEPARTMENT OF CHEMISTRY/UNIVERSITY OF SOUTHERN CALIFORNIA

Many of these lines were found to be of other carbocations, such as $CH_3^+$, $C_2H_3^+$, $C_2H_2^+$, and $CH_2^+$ [for a review, see (*8*)]. Each time I proudly reported these discoveries, Olah responded, "impressive, but what about $CH_5^+$?" After "weeding out" those thousands of understood spectral lines, the remaining messy spectrum was undecipherable, and the 900 lines of $CH_5^+$ were reported without assignment (*1*). Even purely empirical attempts at finding some regularity of the spectrum were not successful.

Asvany *et al.* have been able to determine the energy separation between several pairs of lowest levels using the action spectroscopy invented by Schlemmer and Gerlich (*9*). The proton affinity of $CH_4$ (5.72 eV) is slightly greater than that of $CO_2$ (5.68 eV) so the reaction $CH_5^+ + CO_2 \rightarrow CH_4 + CO_2H^+$ is endothermic. Addition of a resonant 3.3-μm (0.37 eV) laser photon makes this reaction exothermic. Thus, they can do spectroscopy by counting $CO_2H^+$ ions rather than photons. This paradigm shift from photon- to ion-counting spectroscopy has increased the sensitivity—instead of needing $10^{13}$ ions, $10^3$ $CH_5^+$ suffice. Also, trapped ions can be cooled to cryogenic temperature, which leads to a 100 times increase in accuracy.

**"As in Olah's chemistry on Earth, $CH_5^+$ is pivotal for producing hydrocarbons in space... I anticipate that this enfant terrible will be caught in interstellar space far ahead of its theoretical understanding..."**

The 2897 lines observed by Asvany *et al.* at 10 K demonstrate the complexity of the $CH_5^+$ spectrum. In contrast, $CH_4$ at 10 K has only four rotational levels with quantum number $J < 3$ populated and only 10 transitions can be observed. The 300 times increase in spectral density from $CH_4$ to $CH_5^+$ is caused by proton scrambling and inversion motion. Rotational assignments could be made that differ from those they reported, but these are minor details—the CoDiff values are correct and the key to advancing our understanding.

In spite of the complexity, each quantum level can be specified by using the total proton spin quantum number and the parity (*10*). The scrambling of the five protons with spin 1/2 produces a total nuclear spin angular momentum $I$ according to the formula

$$[D_{1/2}]^5 = D_{5/2} + 4D_{3/2} + 5D_{1/2}$$

This formula means that each of the levels of $CH_5^+$ have $I = 5/2$ ($A_1$), 3/2 ($G_1$), or 1/2 ($H_1$), and the number of levels are in the ratio of 1:4:5 and the CoDiffs 1:16:25. Each level also has a definite parity of + or – and their numbers are equal. The CoDiffs reported by Asvany *et al.* are for levels with $I = 3/2$ and 1/2 and the same parity. The next step will be to find CoDiffs with different parity, which the authors note could be tackled by applying their method for far-infrared spectroscopy.

The results by Asvany *et al.* put the experiment far ahead of the theory. To date, Wang and Carrington's computation (*11*), based on the potential energy surface (PES) of Jin *et al.* (*12*), seems to be the only frontal attack to this problem, but it does not include rotation. A brute-force variational calculation of the five protons with an accurate PES may be the way to solve this problem. Such treatment has been successful for $H_3^+$, but the formalism and computation will be much more demanding for a five-proton system.

As in Olah's chemistry on Earth, $CH_5^+$ is pivotal for producing hydrocarbons in space. The lines list by Asvany *et al.* suffices for detecting interstellar $CH_5^+$, but we badly need strongest lines for $I = 5/2$ ($A_1$). The classical $CH_3^+$ ion is yet to be detected, so detection of the nonclassical $CH_5^+$ will be difficult but worth a try. Once the far-infrared transitions are observed, including $I = 5/2$ levels, more sensitive observational techniques can be used. I anticipate that this enfant terrible will be caught in interstellar space far ahead of its theoretical understanding, which will take at least a few more decades. ■

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STATISTICS

# What is the question?

## Mistaking the type of question being considered is the most common error in data analysis

By **Jeffery T. Leek** and **Roger D. Peng**

Over the past 2 years, increased focus on statistical analysis brought on by the era of big data has pushed the issue of reproducibility out of the pages of academic journals and into the popular consciousness (*1*). Just weeks ago, a paper about the relationship between tissue-specific cancer incidence and stem cell divisions (*2*) was widely misreported because of misunderstandings about the primary statistical argument in the paper (*3*). Public pressure has contributed to the massive recent adoption of reproducible research tools, with corresponding improvements in reproducibility. But an analysis can be fully reproducible and still be wrong. Even the most spectacularly irreproducible analyses—like those underlying the ongoing lawsuits (*4*) over failed genomic signatures for chemotherapy assignment (*5*)—are ultimately reproducible (*6*). Once an analysis is reproducible, the key question we want to answer is, "Is this data analysis correct?" We have found that the most frequent failure in data analysis is mistaking the type of question being considered.

Any specific data analysis can be broadly classified into one of six types (see the figure). The least challenging of these is a descriptive data analysis, which seeks to summarize the measurements in a single data set without further interpretation. An example is the United States Census, which aims to describe how many people live in different parts of the United States, leaving the interpretation and use of these counts to Congress and the public.

An exploratory data analysis builds on a descriptive analysis by searching for discoveries, trends, correlations, or relationships between the measurements to generate ideas or hypotheses. The four-star planetary system Tatooine was discovered

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