

compartmentalize this machinery in a water-in-oil droplet. Their IVC system preserves the cell's means of linking DNA to protein, and then adds on *in vitro* chemistry to create the evolutionary pressure. At the other extremes are completely synthetic encoded systems⁷⁻⁹, or solutions that seek to expand the chemistry carried out by the cell¹⁰. The advantage of a completely synthetic system may be that it can go beyond the chemistry that can be synthesized or tolerated by the cell, although the range of chemistry naturally carried out by the cell is awfully impressive.

The field of directed evolution is in a vibrant phase^{11,12}. Beyond that, this tinkering with cells

will provide useful technologies for genomics and biomedical research, and will inspire thinking about what might be synthesized to recapitulate the functions of the cell and how the cell might be co-opted for new functions^{13,14}. ■

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1. Knowles, J. R. *Science* **236**, 1252-1258 (1987).
2. Shaner, N. C., Steinbach, P. A. & Tsien, R. Y. *Nature Meth.* **2**, 905-909 (2005).
3. Aharoni, A., Amitai, G., Bernath, K., Magdassi, S. & Tawfik, D. S. *Chem. Biol.* **12**, 1281-1289 (2005).

4. Mastrobattista, E. *et al. Chem. Biol.* **12**, 1291-1300 (2005).
5. Arnold, F. H. *Nature* **409**, 253-257 (2001).
6. Binz, H. K., Amstutz, P. & Pluckthun, A. *Nature Biotechnol.* **23**, 1257-1268 (2005).
7. Sculimbrene, B. R. & Miller, S. J. *J. Am. Chem. Soc.* **123**, 10125-10126 (2001).
8. Gartner, Z. J. *et al. Science* **305**, 1601-1605 (2004).
9. Halpin, D. R. & Harbury, P. B. *PLoS Biol.* **2**, e174 (2004).
10. Lin, H., Tao, H. & Cornish, V. W. *J. Am. Chem. Soc.* **126**, 15051-15059 (2004).
11. Yoshikuni, Y., Ferrin, T. E. & Keasling, J. D. *Nature advance online publication* doi:10.1038/nature04607 (2006).
12. Park, H. S. *et al. Science* **311**, 535-538 (2006).
13. Szostak, J. W., Bartel, D. P. & Luisi, P. L. *Nature* **409**, 387-390 (2001).
14. Benner, S. A. & Sismour, M. *Nature Rev. Genet.* **6**, 533-543 (2005).

MOLECULAR PHYSICS

Recombination cool and fast

Benjamin J. McCall

Molecular physicists and astrophysicists alike would like to know how fast the H_3^+ molecular ion recombines with electrons. Fast, seems to be the answer — with an awkward consequence for the astrophysicists.

Every schoolchild knows that, like opposing poles of a magnet, opposite charges attract. But what happens when charged bodies are small enough that the rules of quantum mechanics come to bear, for instance when an electron and a positively charged molecule attract? Here, the situation is more complicated: even the reaction between an electron and the simplest polyatomic molecule, H_3^+ (which can be thought of as a hydrogen molecule, H_2 , with an extra proton, H^+) has puzzled both theorists and experimentalists for decades. In a contribution to *Physical Review Letters*, Kreckel *et al.*¹ describe an ingenious experiment that provides further elucidation of the speed of this fundamental reaction.

When an electron approaches a singly charged positive ion (call it X^+), both bodies experience an attraction that accelerates them and causes them to collide. They can recombine to form neutral X, provided that the extra kinetic energy that they have gained by being accelerated can somehow be removed. For macroscopic objects, this is not generally a problem: friction dissipates the energy. At the quantum-mechanical level, however, this cannot happen. If X^+ is the ion of a single atom, energy can be lost only by emitting a photon, a slow process that seldom happens during the short time a collision takes. In most such collisions, the ion and electron fly away from each other again. If X^+ is a molecular ion, however, there is a much more efficient option: the molecule can break apart following recombination with the electron, and the resulting neutral fragments can carry away kinetic energy. This is the process known as dissociative recombination.

The H_3^+ ion assumes an important role in

astrophysics as the first link in a chain of chemical reactions in interstellar clouds through which most of the molecules found in interstellar space form (Fig. 1). Interstellar clouds were recently seen to contain much more H_3^+ than expected², bringing the persistent enigma of its recombination rate back to the fore. Exactly how the dissociative recombination of H_3^+ works was explained

theoretically only recently^{3,4}, and, starting in 1973, many experimental measurements have yielded drastically differing values for the rate at which it occurs⁵.

H_3^+ is produced in ionized gases known as plasmas. Different plasma conditions will lead to different degrees of vibrational and rotational excitation of the H_3^+ ions, perhaps accounting for some of the variation in the experimental recombination rates. If the rate of recombination were lower than assumed, especially at the lower temperatures of interstellar space, the overabundance of H_3^+ in diffuse clouds could be easily explained. But without accurate values for the rate, further progress in understanding the mystery of H_3^+ abundance is impossible.

Hence the efforts to understand dissociative recombination in terrestrial laboratories. In order to best simulate the conditions in

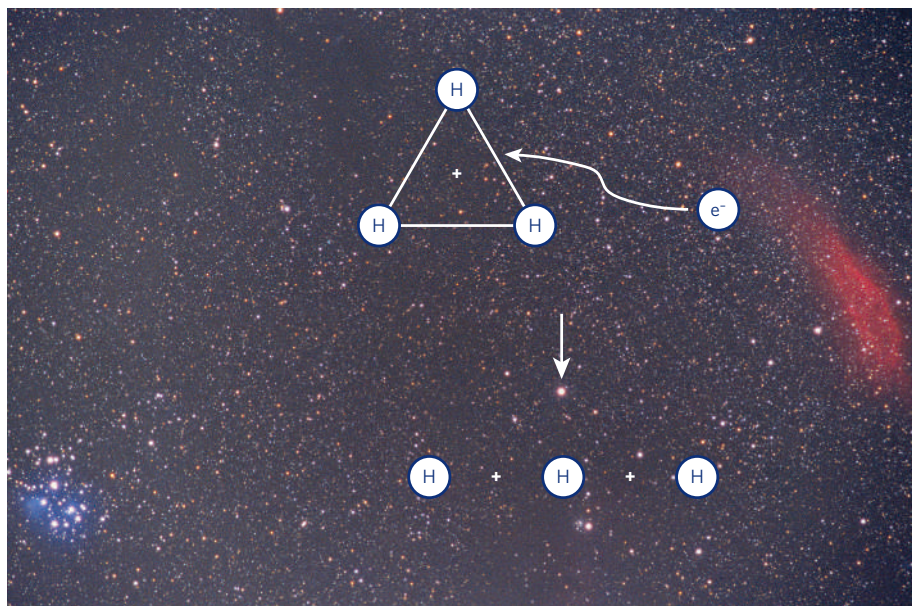


Figure 1 | Cosmic mystery. When an electron and a positively charged H_3^+ ion meet, three separate neutral hydrogen atoms are formed in a process known as dissociative recombination. The image is overlaid on a wide-field image of the Perseus region: the bright stars at bottom left are the Pleiades, or Seven Sisters, and the red region to the right is the California nebula. The bright star at the arrow's head is ζ Persei, where H_3^+ has been observed in unexpectedly high abundance². The results of Kreckel *et al.*¹ imply this cannot be due to slow dissociative recombination. The most likely explanation is instead an enhanced rate of ionization by cosmic rays.

interstellar space, the ions in the laboratory must be cooled to a state of minimum rotation and vibration. Vibrational cooling was first achieved using the CRYRING storage ring in Stockholm, Sweden, by injecting mass-selected ions into an accelerator, and allowing them to relax to their vibrational ground state⁶. Achieving a low rotational temperature is more difficult: H_3^+ cannot cool rotationally by emitting radiation⁷, so the ions must be prepared in a rotationally cold state before they are injected into the storage ring. At CRYRING, this is done by sparking a discharge to ionize a jet of hydrogen gas as it expands through an opened valve into the vacuum of the storage ring and cools⁸. Once the H_3^+ ions are in both their vibrational and rotational ground states, an electron beam travelling at a well-defined velocity is merged with the ion beam, and dissociative recombination occurs. The neutral fragments produced are counted as a function of the relative velocity of the two beams, yielding the 'spectrum' of the rate of recombination as a function of the collision energy.

Kreckel and colleagues' experiments¹, performed at the Test Storage Ring (TSR) in Heidelberg, Germany, follow a similar scheme, but make two improvements over the previous studies. First, they produced their electron beam with a newly developed cryogenic photocathode, allowing more precise control of the ion–electron collision energy and so higher resolution in the dissociative-recombination spectrum. Second, they used a new type of ion source, called a radiofrequency multipole ion trap. In such a trap, H_3^+ ions are stored before injection into the ring at low temperature in the presence of helium gas. The large number of collisions of the ions with the helium means that the rotational energy of the ions can be transferred to the helium, thus ensuring that the ions are rotationally cold. (In contrast, with the expanding jet source used at CRYRING, a small fraction of ions may remain rotationally warm.)

The result of the TSR experiment is in excellent agreement with the CRYRING results, and, thanks to its higher resolution, reveals the fine detail of the spectrum more clearly. It confirms that the rate of dissociative recombination is fast under cool, interstellar conditions. Improved theoretical calculations^{3,4} also yield a rate that agrees well with both experiments. Some minor discrepancies remain, but the general concord implies that the long-standing enigma of the rate of H_3^+ recombination might finally be resolved. If so, the onus is back on the astrophysicists: how can the large observed abundance of H_3^+ in diffuse clouds be explained if recombination is so fast? One likely solution would seem to be enhanced production of H_3^+ through ionization by cosmic rays².

Kreckel and colleagues' results¹ do contain an intriguing twist. At the low temperatures of their measurements (and of the interstellar medium), H_3^+ exists almost entirely in its two lowest rotational states, which have a total

nuclear spin of 3/2 (ortho- H_3^+) and 1/2 (para- H_3^+). But by using para- H_2 in their ion source, and thus enhancing the ratio of para- to ortho- H_3^+ , the authors saw a marked difference in the rate of dissociative recombination at low energies. Unfortunately, they were not able to measure the degree of the para- H_3^+ enhancement, and because of the nature of their ion source, it is probably not very large. Future experiments with pure para- H_3^+ would be highly desirable to elucidate the difference in the rate of recombination between the two states. That would indeed represent the first dissociative recombination measurement of a single quantum state. ■

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1. Kreckel, H. *et al. Phys. Rev. Lett.* **95**, 263201 (2005).
2. McCall, B. J. *et al. Nature* **422**, 500–502 (2003).
3. Kokouline, V., Greene, C. H. & Esry, B. D. *Nature* **412**, 891–894 (2001).
4. Kokouline, V. & Greene, C. H. *Phys. Rev. A* **68**, 012703 (2003).
5. Larsson, M. *Phil. Trans. R. Soc. Lond. A* **358**, 2433–2444 (2000).
6. Larsson, M. *et al. Phys. Rev. Lett.* **70**, 430–433 (1993).
7. Kreckel, H. *et al. New J. Phys.* **6**, 151 (2004).
8. McCall, B. J. *et al. Phys. Rev. A* **70**, 052716 (2004).

NEUROBIOLOGY

How fast can you go?

Laura N. Borodinsky

Rhythmic activities such as walking need tight coordination. In mice, pace is tweaked by a specific set of spinal-cord neurons that, surprisingly, make the animals walk faster by inhibiting the underlying circuit.

Watch your step — walking may seem simple, but is actually quite a complex task. As with other rhythmic motor behaviours (breathing or swallowing, say), locomotion relies on a finely tuned neuronal network that is headquartered in the spinal cord^{1,2}. The ensemble of spinal neurons that generates a coordinated rhythmic activity is known as a central pattern generator. The rhythm and periodicity of this network determines movement features such as the alternation between left and right, or the speed of walking, jumping or swimming³. Understanding how this circuit operates and the specific roles of the different neurons that participate in it is difficult, but Gosgnach and colleagues⁴ have taken up the challenge. On page 215 of this issue, they report that the activity of a group of spinal-cord neurons controls the speed of locomotor behaviour in the mouse.

Neuronal circuits are formed by a network of interconnected excitatory and inhibitory neurons. In a very simplistic model, the former group switches the circuit on and the latter turns it off. Gosgnach *et al.* studied the role of a subclass of inhibitory spinal neurons known as V1 neurons, which are thought to be part of the central pattern generator. The researchers used detailed information about the gene-regulatory factors that underlie the development and specialization of these neurons, to generate mutant mice in which V1 neurons were either eliminated or silenced acutely during the experiment, and examined the changes in locomotor activity.

Counterintuitively, they found that removing the inhibition caused by V1 neurons slows the speed of the locomotor rhythm. Mutant

mice lacking V1 neurons are unable to walk fast, but they can maintain normal motor behaviour at a slower pace. The authors demonstrate that this is because motor neurons connecting the spinal cord to the muscle are not sufficiently inhibited, because connections from the V1 neurons are missing in the mutant animals. The activity of motor neurons needs to be interleaved with precise periods of silence to generate a faster pace. These results underscore the value of inhibition in the nervous system: the delicate balance and fine-tuning of neuronal activity set by inhibitory connections is not only important in quieting down the system, but can also change core features of nervous-system function.

Even though Gosgnach and colleagues' mice slow down, other parameters of their locomotor activity remain intact, such as the alternation of left and right limbs necessary for coordinated stepping. As the authors showed previously⁵, a different class of spinal neurons (V0) is responsible for left–right coordination. In mutant mice lacking V0 neurons, the left and right motor neurons fire at the same time, rather than alternating. These are significant insights into how the work is distributed among the vast collection of spinal neurons that make up the central pattern generator.

How are the circuits of the central pattern generator established? When exploring the formation of circuits, scientists have focused on two principal alternative theories. One of these proposes that a genetically driven programme predetermines the identity of the neurons that participate in a given circuit and dictates how and between which of them connections are made⁶. The second proposes that