presentation. Autophagy is an essential cellular degradation pathway that recycles organelles and proteins to maintain cellular ‘fitness’[12]. It can act selectively through the binding of a specific receptor to a ‘cargo’ (for example, a protein or organelle) that has been targeted for destruction by being marked with a tag, such as the protein ubiquitin. A complex of receptor and cargo is enveloped in a lipid membrane to form a vesicle called an autophagosome, which fuses with an organelle known as a lysosome to form an autolysosome (Fig. 1). The cargo then undergoes enzyme-mediated digestion in the autolysosome and its contents are recycled for use in the cell.

Yamamoto *et al.* report that, remarkably, in pancreatic cancer cells, most MHC class I molecules do not exist on the cell surface, but instead are found in autophagosomes and autolysosomes. The authors identified an autophagy-associated receptor called NBR1 as being responsible for targeting MHC class I molecules to the autophagy machinery. Furthermore, they found that if autophagy was inhibited in mice, by drugs such as chloroquine or through genetic engineering, this restored the surface expression of MHC class I molecules, thereby enhancing antigen presentation. In mouse models of pancreatic cancer, autophagy inhibition resulted in an influx of cytotoxic T cells to the tumour microenvironment, and if the animals also received checkpoint blockade therapy, a robust antitumour immune response was generated.

Increased autophagy has been known for around a decade[13] to be a metabolic requirement for pancreatic cancer, but only now has a connection been made to the immune evasion of tumour cells. Thus far, clinical trials targeting autophagy in pancreatic cancer have relied on testing the antimalarial drug hydroxychloroquine (chloroquine and hydroxychloroquine are related molecules), which blocks one of the final steps in autophagy. Other drug candidates, directed at earlier components of the autophagy machinery, are in the pipeline. Early trials of hydroxychloroquine demonstrated only modest results, but there has been a resurgence of interest in combinatorial treatment approaches after evidence from animal models that, if signalling mediated downstream of mutant Ras by the enzyme MAP kinase is inhibited, pancreatic cancer cells become strongly dependent on autophagy for their survival[14,15].

Yamamoto and colleagues’ work will almost certainly lead to further additions to the compendium of autophagy-targeted clinical trials of pancreatic cancer treatments. Discoveries in the fields of autophagy and immunotherapy were, respectively, recognized by the Nobel Prize in Physiology or Medicine in 2016 and 2018. This new finding represents an unprecedented opportunity for the convergence of these two areas of study, in efforts to improve therapies for pancreatic cancer.

Anirban Maitra is in the Sheikh Ahmed Center for Pancreatic Cancer Research, University of Texas MD Anderson Cancer Center, Houston, Texas 77005, USA.
e-mail: amaitra@mdanderson.org

1. Yamamoto, K. *et al. Nature* **581**, 100–105 (2020).
2. Wolchok, J. *Cell* **175**, 1452–1454 (2018).
3. O’Reilly, E. M. *et al. JAMA Oncol.* **5**, 1431–1438 (2019).
4. Balachandran, V. P., Beatty, G. L. & Dougan, S. K. *Gastroenterology* **156**, 2056–2072 (2019).
5. Yao, W., Maitra, A. & Ying, H. *EBioMedicine* **53**, 102655 (2020).
6. Whittle, M. C. & Hingorani, S. R. *Gastroenterology* **156**, 2085–2096 (2019).
7. Pommier, A. *et al. Science* **360**, eaao4908 (2018).
8. Gettinger, S. *et al. Cancer Discov.* **7**, 1420–1435 (2017).
9. Grasso, C. S. *et al. Cancer Discov.* **8**, 730–749 (2018).
10. Waddell, N. *et al. Nature* **518**, 495–501 (2015).
11. Ryschich, E. *et al. Clin. Cancer Res.* **11**, 498–504 (2005).
12. Santana-Codina, N., Mancias, J. D. & Kimmelman, A. C. *Annu. Rev. Cancer Biol.* **1**, 19–39 (2017).
13. Yang, S. *et al. Genes Dev.* **25**, 717–729 (2011).
14. Bryant, K. L. *et al. Nature Med.* **25**, 628–640 (2019).
15. Kinsey, C. G. *et al. Nature Med.* **25**, 620–627 (2019).

Precision measurements

Mass spectrometry for future atomic clocks

Marianna S. Safronova

Highly charged ions could form the basis of the next generation of ultra-precise clocks, using electronic transitions in the ions as the ‘pendulum’. An ingenious method for characterizing such transitions has been reported. **See p.42**

Atomic clocks, which use transitions between the energy levels of electrons in atoms as a reference for their timekeeping mechanism, are the world’s most accurate clocks — they will not lose one second during the lifetime of the Universe[1]. This means that they can be used in ultra-precise measurements to probe some of the fundamental postulates of modern physics. Clocks based on highly charged ions (HCIs; atoms from which many electrons have been removed) are predicted to have even more sensitivity in these investigations[2]. However, the development of such clocks is hampered by the difficulty of detecting suitable transitions in HCIs.

> **“The authors used Einstein’s principle of energy–mass equivalence to convert a mass measurement into an energy measurement.”**

On page 42, Schüssler *et al.*[3] report that they have measured a long-lived, excited electronic state in a highly charged rhenium ion using the mass difference of the ion in its ground and excited states. This non-destructive, direct determination of an electronic excitation in an HCI will aid the discovery of HCI transitions that would be suitable for use in a clock.

To build a clock, one needs a periodic event whose frequency acts as a reference for timekeeping. Electronic transitions in atoms are perfect natural oscillators for this purpose. An ultra-stable laser must be tuned to the exact frequency of the atomic transition to drive the oscillation, much as a musical instrument must be tuned to produce the right tone.

Can just any atomic transition be used? No — suitable transitions are hard to come by. The best transitions start from the lowest energy state of an atom (the ground state) and must end up in a long-lived (metastable) excited state. The energy needed to stimulate the transition must also be within the range of tabletop-laser technologies.

Moreover, the atoms must be held in traps, so that their motion is almost completely frozen — in other words, the operation of atomic clocks requires precision manipulation of quantum systems. For this reason, currently available clocks use transitions either in electrically neutral atoms or in ions produced by removing one electron from an atom, because these systems are the most amenable to precision quantum control.

Substantial advances have been made in studies of HCIs, and all the technologies required to make a clock using such an ion were demonstrated only this year[4]. However, progress is hindered by the difficulty in using conventional atomic spectroscopy to identify and measure transitions suitable for use in clocks — the characteristics of such transitions mean that they are, by definition, very weak (the probability of the transition occurring

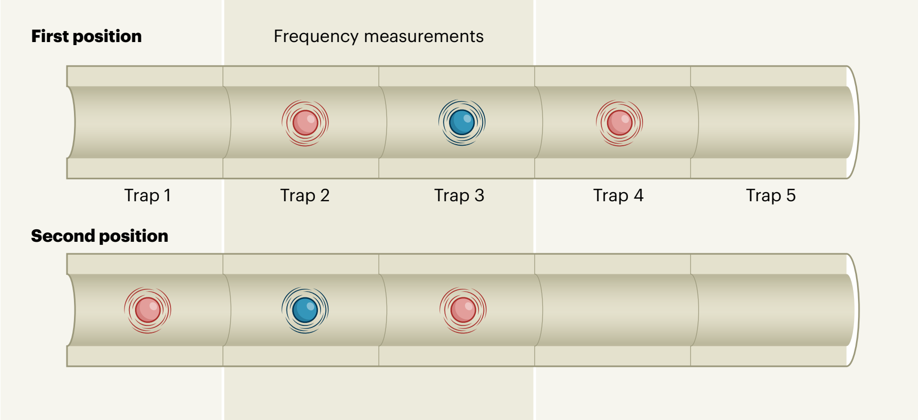

Figure 1 | Mass measurements of a highly charged ion. Schüssler *et al.*[3] used an instrument called PENTATRAP, which consists of five stacked ion traps, to determine how the mass of the rhenium ion Re^{29+} differs in its ground state (blue ions) and in a metastable excited state (red ions). The authors captured three ions in their first position (traps 2–4) at 4 kelvin, and simultaneously measured the cyclotron frequency – the frequency of an ion's motion in a magnetic field – of the ions in traps 2 and 3. All three ions were then shifted to their second position, so that the ions in traps 2 and 3 had different states from the ones that were trapped in the first position. The cyclotron frequencies of the ions in those traps were measured, and the whole sequence was repeated many times. From the ratio of cyclotron frequencies of ions in the two different states, the authors determined the associated difference in ion mass, and thus the change of energy that occurs when a Re^{29+} ion transitions between states. Such a measurement would be difficult using conventional atomic spectroscopy.

is small). Schüssler *et al.* therefore used a completely different and ingenious method to measure the energy change that occurs during a weak transition in a highly charged rhenium ion (Re^{29+}): they used Einstein's famous principle of energy–mass equivalence ($E = mc^2$) to convert a mass measurement into an energy measurement.

The basic idea is to trap a single ion in a Penning trap, a device that confines charged particles using magnetic and electric fields. The mass of an ion in a Penning trap can be determined by measuring the frequency of the ion's motion in a magnetic field (the cyclotron frequency). The binding energy of an atom or ion – the energy required to break the atom into its free electrons and a nucleus – is different in an excited metastable state from that in the ground state. The mass therefore also changes, which, in turn, alters the cyclotron frequency.

In their experiments, Schüssler *et al.* measured the ratio (R) of the cyclotron frequency of Re^{29+} in the ground state and the metastable state. Because the difference in energy of the two states in Re^{29+} is extremely small compared with the total energy of the ion, the precision of the measurement needs to be extraordinarily high. The authors measured R to a precision of 10^{-11}, using a device known as PENTATRAP.

PENTATRAP consists of a stack of five Penning traps cooled to a temperature of 4 kelvin (Fig. 1). Traps 2 and 3 are used to measure cyclotron frequencies, whereas traps 1 and 4 are used to store ions. Trap 5 was not used in the current experiments, but will allow monitoring of fluctuations in the magnetic field and in other experimental variables in the future.

The authors loaded three ions into the innermost traps, so that the ions in traps 2 and 4 were in the same state (either the metastable state or the ground state), and the ion in trap 3 was in the alternative state. First, they measured the cyclotron frequencies of the ions in traps 2 and 3 simultaneously. They then moved the three ions up by one trap, effectively swapping the states of the ions in traps 2 and 3 (the states of the ions did not change, only their positions; Fig. 1), and simultaneously measured the cyclotron frequencies of those ions. The three ions were moved back down by one trap, and the sequence began again. Overall, the electronic states in traps 2 and 3 were repeatedly swapped, and simultaneous measurements were taken after each swap.

This experimental procedure, combined with the design of the PENTATRAP device, suppresses the effect of magnetic-field variations on R, thus allowing R to be determined with high accuracy. The energy difference between the ground and excited states can then be calculated using R and the ion mass in a variant of Einstein's equation; the actual mass of the ion needs to be known only to a precision of 10^{-4}.

This first demonstration of the method opens up exciting possibilities for measuring the transition energies in HCIs that are difficult to measure using conventional approaches. Moreover, the energy change measured by Schüssler and colleagues is in excellent agreement with that predicted from the authors' advanced theoretical calculations. This agreement demonstrates that theory can be used to predict the transition energies in HCIs, thereby facilitating the discovery of more transitions.

The transition energy measured in the current work corresponds to a frequency that lies outside the range of lasers that can be used in a clock. However, the authors note that it should be possible to use their method to measure transitions that have lower frequencies suitable for clock development in the near future.

Clocks based on HCI transitions are particularly attractive because they could be used in stringent tests that are sensitive enough to detect physics beyond the standard model of particles and interactions – such as variations of fundamental physical constants and violations of Lorentz invariance[2] (a cornerstone of physics that acts as the mathematical foundation for Einstein's special theory of relativity). Such clocks would also be particularly sensitive to the effects of ultralight dark matter[2,5], one of the candidates for the 'missing' matter in the Universe. Tremendous progress in the control of HCIs has been made in the past few years[2,4,6], paving the way towards these applications. The precision mass spectrometry enabled by PENTATRAP will also have other valuable applications[7], such as in tests of the energy–mass equivalence principle, experimental determinations of the upper limits of the mass of neutrino particles, and tests of quantum electrodynamics, the theory that describes the interactions between particles and light.

Marianna S. Safronova is in the Department of Physics and Astronomy, University of Delaware, Newark, Delaware 19716, USA.
e-mail: msafrono@udel.edu

1. Brewer, S. M. *et al. Phys. Rev. Lett.* **123**, 033201 (2019).
2. Kozlov, M. G., Safronova, M. S., Crespo López-Urrutia, J. R. & Schmidt, P. O. *Rev. Mod. Phys.* **90**, 045005 (2018).
3. Schüssler, R. X. *et al. Nature* **581**, 42–46 (2020).
4. Micke, P. *et al. Nature* **578**, 60–65 (2020).
5. Safronova, M. S. *et al. Rev. Mod. Phys.* **90**, 025008 (2018).
6. Schmöger, L. *et al. Science* **347**, 1233–1236 (2015).
7. Dilling, J., Blaum, K., Brodeur, M. & Eliseev, S. *Annu. Rev. Nucl. Part. Sci.* **68**, 45–74 (2018).