different in the soluble and particulate enzymes. But the variable activity and copper content of different preparations of pMMO[5,7–10], the presence of iron in almost all of those preparations[5], and the looming precedent of di-iron sMMO, left room for doubt.

The X-ray crystal structures[11,12] of pMMOs from two different methanotrophs did little to allay this uncertainty. The enzyme preparations used for these studies had minimal catalytic activity, precluding insight into the active form. Moreover, the structures revealed actual or potential binding sites for three different metal ions in four different locations (Fig. 1): di-copper and mono-copper centres in the amino-terminal domain of the primarily extramembrane subunit, pmoB; a site in the intermembrane subunit pmoC that contained either a single copper or zinc ion, but which was suggested to be capable of housing a di-iron cluster[12,13]; and, within the other intermembrane subunit, pmoA, a cluster of hydrophilic amino-acid residues that was later suggested to be the site of a tri-copper centre[7].

The simmering controversy came to a boil when Münck and co-workers[13] provided spectroscopic evidence that their relatively active preparations of pMMO contained a significant quantity of a di-iron cluster similar to the cofactor of sMMO, and, more compellingly, that the enzyme activity of these preparations might correlate with their iron content. Perhaps, these authors suggested, pMMO and sMMO both use di-iron cofactors.

Balasubramanian *et al.*[1] have now resolved the copper-versus-iron controversy using a reductionist approach. They first extracted all the metal ions from intact *Methylococcus capsulatus* membranes that contained active pMMO, thereby inactivating the enzyme. When they then added three equivalents of copper ions per molecule of enzyme back to the metal-free samples, they observed that about 90% of the original MMO activity was regenerated. But when they added iron ions, the enzyme was not reactivated.

This copper-specific reactivation seemed consistent only with a copper cofactor, but the authors sought more proof and deeper insight. Anticipating that the metals in the pmoB domain constitute the active site of the enzyme, they created a soluble, stand-alone version of this subunit that lacked the transmembrane helices of the natural protein and that had its extramembrane amino- and carboxy-terminal domains joined by a short peptide linker. They expressed this protein (known as spmoB) in *Escherichia coli*, a bacterium devoid of MMO activity. The protein failed to fold properly in *E. coli*, but, stunningly, Balasubramanian *et al.* were able to refold the isolated protein into its active form.

Again, the team found that copper was required for spmoB to be active; iron was no substitute. When the authors reconstituted misfolded spmoB in the presence of copper ions, they found that the protein acquired about three copper ions per molecule, consistent with the idea that pmoB contains a mono-copper site and a di-copper site in the native enzyme[12]. The authors also obtained spectroscopic data to confirm that a cluster of at least two copper ions assembled both in reactivated membranes and in refolded spmoB. These results showed that the pmoB subunit is sufficient for the methane-oxidation activity of pMMO, and that copper atoms are responsible for this activity.

Pushing one step further, Balasubramanian *et al.*[1] asked whether the di-copper centre, the mono-copper centre, or both centres of spmoB are required for methane oxidation. To do this, they expressed the N- and C-terminal domains of spmoB separately, and found that the N-terminal domain (spmoBd1) could take up only about two copper ions in the refolding procedure. This suggests that the mono-copper site is lost when spmoBd1 is isolated alone, in accord with evidence[12] that the mono-copper site lies at the interface of spmoBd1 and the C-terminal domain (spmoBd2). Remarkably, spmoBd1 was found to retain detectable — albeit low — MMO activity, strongly implicating its di-copper site as the site of methane oxidation. Consistent with this conclusion, neither isolated spmoBd2, nor variants of spmoB that lacked the ability to bind a di-copper cluster, showed detectable MMO activity.

Sceptics may point out that Balasubramanian and colleagues' spmoB constructs[1] oxidize methane much less rapidly than preparations of authentic pMMO. Does this cast doubt on the team's conclusions? It seems almost inconceivable that the authors could somehow have assembled a cofactor capable of such a difficult reaction in a site that was not meant to contain such a cofactor. It seems equally improbable that pMMO would have more than one site of methane oxidation. The fact that these proteins have any activity at all is therefore compelling evidence for the authors' conclusions, especially when one considers that they were isolated from a non-methanotrophic organism and so are necessarily free from any possible contaminant capable of methane oxidation (including the other pMMO components).

Indeed, the production of a minimalistic di-copper-binding protein unit and its copper-specific reconstitution to an active MMO[1] is among the starkest evidence imaginable for a di-copper cofactor in pMMO. Moreover, Balasubramanian and colleagues' soluble proteins may now be useful tools for dissecting the mechanism of oxygen activation and methane hydroxylation at a copper centre. Together with recent advances from studies on methane hydroxylation by inorganic catalysts that contain a di-copper unit[14], these tools promise to bring our understanding of copper-mediated methane oxidation to the level achieved for the better studied di-iron sMMO and relevant inorganic models. ■

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1. Balasubramanian, R. *et al. Nature* **465,** 115–119 (2010).
2. Hakemian, A. S. & Rosenzweig, A. C. *Annu. Rev. Biochem.* **76,** 223–241 (2007).
3. Kovaleva, E. G., Neibergall, M. B., Chakrabarty, S. & Lipscomb, J. D. *Acc. Chem. Res.* **40,** 475–483 (2007).
4. Merkx, M. *et al. Angew. Chem. Int. Edn* **40,** 2782–2807 (2001).
5. Rosenzweig, A. C. *Biochem. Soc. Trans.* **36,** 1134–1137 (2008).
6. Wilkinson, B. *et al. J. Am. Chem. Soc.* **118,** 921–922 (1996).
7. Chan, S. I. *et al. Angew. Chem. Int. Edn* **46,** 1992–1994 (2007).
8. Chen, K. H.-C. *J. Chin. Chem. Soc.* **51,** 1081–1098 (2004).
9. Ngyuen, H.-H. T. *et al. J. Am. Chem. Soc.* **118,** 12766–12776 (1996).
10. Yu, S. S. *et al. Biochemistry* **46,** 13762–13774 (2007).
11. Hakemian, A. S. *et al. Biochemistry* **47,** 6793–6801 (2008).
12. Lieberman, R. L. & Rosenzweig, A. C. *Nature* **434,** 177–182 (2005).
13. Martinho, M. *et al. J. Am. Chem. Soc.* **129,** 15783–15785 (2007).
14. Woertink, J. S. *et al. Proc. Natl Acad. Sci. USA* **106,** 18908–18913 (2009).

NONLINEAR DYNAMICS

Optoelectronic chaos

Laurent Larger and John M. Dudley

Optoelectronic circuits with delayed feedback provide a convenient bench-top platform to study a wide range of nonlinear dynamic systems, from ultrastable clocks to complex chaotic devices.

Every time we turn on a computer we are reminded that nothing happens instantaneously. All physical systems exhibit a finite time delay between the moment we provide a stimulus and the moment the system returns a response. In many situations, the response also depends nonlinearly on the input, such that the evolution of the system in the present depends very sensitively on its state in the past. Such nonlinear time-delay systems can display complex dynamic behaviour, but in fact they are ubiquitous in nature and include such examples as predator–prey relationships, cell regulation and neural synchronization.

But somewhat surprisingly, even though nonlinear delay dynamics have wide relevance in both fundamental science and technology, there are few laboratory systems with which their complexity can be readily studied. Consequently, much of the fundamental science of how nonlinearity and delay interact remains unexplored. Recent years, however, have seen the development of a range of optical systems that have opened up the richness and complexity of nonlinear delay dynamics to systematic experimental study[1–3]. Reporting in *Physical Review Letters*, Callan *et al.*[4] highlight a significant advance in this field by using a type of optoelectronic circuit called an optoelectronic oscillator to study the way in which an external perturbation can switch a nonlinear delay system between stable and chaotic operation.

An optoelectronic oscillator is a system in which the intensity of a continuous-wave laser (one that, in contrast to a pulsed laser, produces a continuous light beam) is modulated by an electronically driven nonlinear optical device before being fed into a long length of optical fibre, which introduces delay (Fig. 1a). After propagation in the fibre, the delayed light is detected electronically, and this output is then used as an input to drive the modulation of the laser once again. The properties of the delayed output thus feed back into the system to modify the input, which in turn generates a new output and so on, in a periodic (oscillatory) manner. This is clearly not a simple system and, indeed, such oscillators exhibit a wide range of rich dynamics[2] (Fig. 1b).

One common application of optoelectronic oscillators is to use the feedback between input and output to force the system to generate a precise repetitive signal that can be used as an ultrastable clock at microwave frequencies for applications such as radar[5]. But optoelectronic oscillators can also be configured to generate chaotic noise-like waveforms over a wide range of frequencies, and it is the novel dynamics of this regime that Callan and colleagues[4] have investigated in their study.

The particular originality here lies in the authors' ability to switch the dynamics of the system in a controlled way between two fundamentally different states. By increasing the amplitude of an applied perturbation (noise from an optical amplifier), they can study the transition from the system's steady state to a mode of broadband chaos. The transition regime is associated with the generation of a series of ultrashort pulses that slowly grow in amplitude and progressively merge until the fully developed chaotic regime is reached. Although the noise characteristics of the generated chaos are subtle, a specific characteristic of the system output is that it is 'featureless'. In other words, there is essentially no residual signature of any periodicity in the output even though the noise is actually generated by a cyclic oscillator.

The authors[4] mention one particular application of their system in distributed sensor networks (used, for example, in automated information gathering in areas such as home energy management and real-time object tracking). In addition, their system could also have real advantages in applications to improve communications security[6], in which alternative chaos-generating nonlinear delay-based systems[7] have shown real promise at data-transmission rates above 10 Gbit s^{-1}.

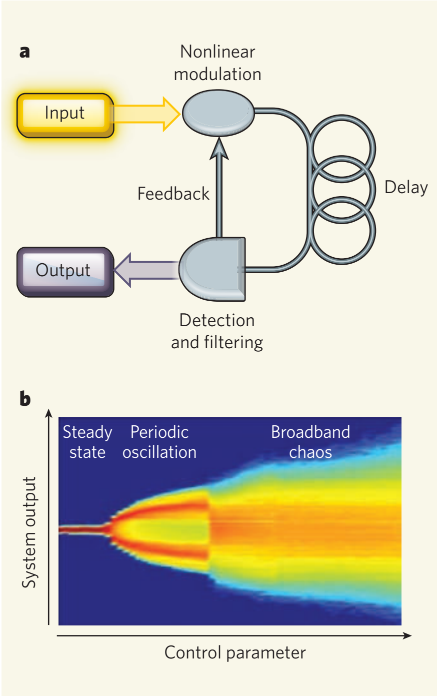

Figure 1 | Working principle and dynamic behaviour of an optoelectronic oscillator. **a**, Generic architecture of the system: the input, the optical signal from a laser, is modulated nonlinearly before propagating in an optical fibre, which provides time delay. Detection and filtering at the fibre output convert the optical signal into an electronic signal, part of which is taken as the output of the system and part of which is fed back to re-drive the nonlinear modulation of the optical input. **b**, The system output is illustrated generically via a bifurcation diagram, which plots the amplitude of the output as a function of a control parameter that can be taken as proportional to the intensity of the input after modulation. Optoelectronic oscillators typically exhibit three dynamic regimes: steady state, which is independent of the control parameter; periodic oscillation, in which two discrete output values (the 'zeros' and 'ones' of a pulse train, for example) are seen; and a broadband chaotic regime. The colour scale represents the amplitude distribution of the output (blue is zero, red is maximum). The originality of Callan and colleagues' study[4] is that the steady-state and chaotic regimes of the system coexist and can be readily switched from one to the other using a small perturbation.

The results will also have a wide impact on the fundamental science of nonlinear dynamics because they highlight how optoelectronic systems provide versatile bench-top tools to study the dynamics of nonlinear delay systems. The advanced technological development of optoelectronic components allows convenient manipulation of almost all the important system parameters, such as delay, nonlinearity, and the form and frequency bandwidth of the dynamic response. In addition, although Callan and colleagues' work considers only one oscillator, a natural extension would involve coupling between several oscillators. This would open up new perspectives for studying synchronization dynamics[8] and neural-network-like approaches for informatics such as the emerging field of reservoir computing[9]. Optoelectronic systems allow researchers to create a 'bench-top nonlinear simulator' of essentially any complex system of interest, and their increasingly widespread use is sure to provide further insights into the science and applications of chaos. ■

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1. Fischer, I. *et al. Phys. Rev. Lett.* **76,** 220–223 (1996).
2. Peil, M. *et al. Phys. Rev. E* **79,** 026208 (2009).
3. Murphy, T. E. *et al. Phil. Trans. R. Soc. A* **368,** 343–366 (2010).
4. Callan, K. *et al. Phys. Rev. Lett.* **104,** 113901 (2010).
5. Yao, X. S. & Maleki, L. *Electron. Lett.* **30,** 1525–1526 (1994).
6. Argyris, A. *et al. Nature* **438,** 343–346 (2005).
7. Lavrov, R. *et al. Phys. Rev. E* **80,** 026207 (2009).
8. Vicente, R. *et al. Proc. Natl Acad. Sci. USA* **105,** 17157–17162 (2008).
9. Maass, W. *et al. Neural Comput.* **14,** 2531–2560 (2002).

HEPATITIS C

An unsuspected drug target

Catherine L. Murray and Charles M. Rice

Infection with hepatitis C is one of the main causes of liver disease, yet there are no broadly effective treatments. Discovery of a potent inhibitor of this virus shows that researchers must think outside the box.

Hepatitis C virus is nasty. Chronic infection with this virus (HCV) can lead to liver disorders such as fibrosis, cirrhosis and hepatocellular carcinoma, and it is a major reason for liver transplantation. Unfortunately, there is no HCV vaccine, and existing therapies are limited, often poorly tolerated and frequently ineffective. Clearly, new strategies to prevent and treat infections with this virus are urgently needed. On page 96 of this issue, Gao *et al.*[1] identify a compound that inhibits HCV replication and come up with a few surprises: this agent is the most potent HCV inhibitor reported so far, and it does not attack the virus's