such microscopy techniques are often carried out on dried samples, and the drying can profoundly affect the structure of the fibres.

What has really been missing from the field is the ability to directly image multi-component self-sorted networks on a longer scale, and show clearly whether the self-sorting is just a local phenomenon or rather that the assembly is truly made of two distinct fibrous networks. The properties of the resulting gel will also greatly depend on how the two networks co-exist; are they present as a genuinely interpenetrated, distinct two-network system, or, for example, does one network template the other? Do the fibres formed from one gelator prefer to entangle with themselves, or is there no discrimination at that step? These are questions that had until now been beyond our capability to answer.

Hamachi and co-workers have mixed two different gelators, both of which are known to form single-component fibrous networks. Together, the gelators also form a fibrous gel. In a similar manner to the characterization of previous systems, techniques such as circular dichroism showed that self-sorting occurs locally. Where the present work goes beyond the level of insight gained previously is through the direct imaging of the two individual networks. By incorporating specific fluorescent molecules that only bind with one of the components, the researchers collected data that clearly showed the formation of an interpenetrated, self-sorted, two-component network (Fig. 1). Using confocal and stimulated emission depletion microscopy, they were able to obtain images with a high resolution (80 nm) — and importantly did so directly in the gel state, so that any drying artefacts could also be ruled out. It is worth noting that the use of these imaging tools also allowed a 3D representation of the networks to be obtained.

This achievement would have been impressive in itself, but the researchers have also gone a step further. Using fluorescence recovery after photobleaching (FRAP) techniques, they were able to show that the fibres behave as fluidly in the two-component gel as they do in their respective single-component counterparts. One set of fibres was found to be more stable than the other. This shows that the two networks are behaving truly independently, and that growing one in the presence of the other does not affect the packing of either building block.

Real-time imaging of the formation of the self-sorted gel showed that the growth of each set of fibres was essentially independent — each forms in the same way as it does in a single-component system. Furthermore, the assembly process could to some extent be controlled by seeding. Adding seeds to the single component system or multi-component system introduces a nucleation site from which self-assembly is initiated. This allows easy visualization, and therefore insight, into the start of the process. The incorporation of OG-BP seeds promoted a more rapid growth for these fibres (normally slower than the Alexa546-cycC_6 ones), hence also showing that the seeds can affect the morphology of the growing network. These experiments illustrate the importance of the initial nucleation on the rate of self-assembly.

This study provides an exciting and useful methodology to better understand and control self-sorting events, which should in turn allow us to work out how to design and prepare specific functional systems. This work brings us a step closer to one day being able to compete with nature's level of complexity. ❐

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References
1. Stupp, S. I. & Palmer, L. C. *Chem. Mater.* **26,** 507–518 (2014).
2. Onogi, S. *et al.*, *Nature Chem.* **8,** 743–752 (2016).
3. Buerkle, L. E. & Rowan, S. J. *Chem. Soc. Rev.* **41,** 6089–6102 (2012).
4. Safont-Sempere, M. M., Fernández, G. & Würthner, F. *Chem. Rev.* **111,** 5784–5814 (2011).
5. Philp D. & Stoddart, J. F. *Angew. Chem. Int. Ed. Engl.* **35,** 1154–1196 (1996).
6. Escuder, B. & Miravet, J. F. *Functional Molecular Gels* (Royal Society of Chemistry, 2013).
7. Raeburn, J. & Adams, D. J. *Chem. Commun.* **51,** 5170–5180 (2015).
8. Sugiyasu, K., Kawano, S.-i., Fujita, N. & Shinkai, S. *Chem. Mater.* **20,** 2863–2865 (2008).

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DNA REACTION NETWORKS

Providing a panoramic view

A quantitative understanding of the functional landscape of a biochemical circuit can reveal the design rules required to optimize the circuit. Now, a high-throughput droplet-based microfluidic platform has been developed which enables high-resolution mapping of bifurcation diagrams for two nonlinear DNA networks.

Fei Wang and Chunhai Fan

DNA base pairing governs the interactions of nucleic acid strands in living systems. Inspired by this naturally occurring phenomenon, researchers have exploited DNA hybridization — which follows the simple Watson–Crick pairing rules — to develop various static nanostructures and dynamic nanodevices for sensing, imaging, therapeutic applications and DNA computation[1–4]. In one notable study Aldeman pioneered the use of DNA for massively parallel computation[3], which holds great promise for information storage, smart drug delivery and even the rewiring of natural signalling pathways *in vivo*. However, the scale, function and complexity required by real-world applications are not satisfactorily met by state-of-the-art DNA computers. This is in part because of the nonlinear and analogue nature of biochemical circuits, which means that the complexity of DNA computation systems is much higher than that of a digital electronic system containing the same number of components. Consequently, our understanding of how a DNA circuit operates remains poor, and the design of these systems is largely empirical[4].

The high predictability of DNA binding thermodynamics enables the design of DNA-based logic gates with reasonable ease[5]. Nevertheless, when a circuit design contains a large number of variables, one has to carefully adjust various parameters and test many combinations to achieve the desired function, which is a rather laborious process and optimization can sometimes seem impossible[6]. The typically available technologies only permit a small number of checkpoints to be monitored in designed DNA circuits, so this often results in counterintuitive output. However, individually analysing each of the involved reactions could enable a quantitative understanding of the whole system and should lead to the rational design of DNA circuits that provide the expected output.

Recent advances in droplet-based microfluidics have shed new light on high-throughput analysis of biochemical networks. In such microfluidic platforms, each droplet carries different conditions, which can be simultaneously imaged to characterize the output of tens of thousands of biochemical reactions in parallel. In 2013 a team led by Yannick Rondelez demonstrated that synthetic DNA oscillators can be encapsulated in microscale water-in-oil droplets without affecting their function[7], which suggested that a biochemical network can be partitioned into micro-vessels to greatly improve throughput. Now, writing in *Nature Chemistry*, Rondelez and co-workers report that droplet microfluidics can be used to map the functional landscapes of two synthetic DNA circuits[8]. For the first time, they visualized the bifurcation diagrams for these two nonlinear, out-of-equilibrium biochemical systems with high resolution and dimensionality, demonstrating the ability to uncover the mechanisms of complex molecular circuits in a quantitative and parallel manner thereby providing a panoramic view of the reaction landscape.

To produce droplets with different chemical compositions of n parameters, Rondelez and co-workers prepared n reaction tubes each containing a different species that carried a spectrally resolvable fluorescent barcode. These tubes were connected to the inlets of a microfluidic chip such that the initial conditions contained within droplets could be reliably controlled by varying the pressure of the tubes. From a bulk reaction volume of 100 μl, one could yield at least 10^4 droplets each day, which were collected and imaged as an ensemble by using a confocal microscope. The throughput was also increased by more than an order of magnitude compared with their previous work[7], providing a practical route to high-resolution mapping of bifurcation diagrams.

The team then employed this droplet-based microfluidic platform to analyse a bistable DNA switch circuit[9] with two ssDNA triggers, α and β, that were self-replicating and mutually inhibiting. By designing a set of DNA hybridization and polymerization/nicking reactions the team were able to monitor the survival or decay of the triggers, which resulted in four equilibrium states. The concentrations of two autocatalytic templates, $\alpha to \alpha$ and $\beta to \beta$, were defined as design parameters. Starting from $\alpha\beta = 10$ or $\alpha\beta = 01$, they generated bifurcation diagrams in the (α,β) space against the parameters $(\alpha to \alpha, \beta to \beta)$ by monitoring the fluorescence change in each channel from the confocal images, which arises from the binding of output strands to the templates. A bistable region where the DNA switch functions as a memory — that is, the device can integrate a transient molecular stimulus into a sustained molecular response[9] — was identified from the superposition of the two mapped diagrams. This shows that by generating a bifurcation diagram it should be possible to design new bistable DNA switches by choosing the levels of parameters from the bistable region, and that this should optimize the functional output.

The researchers then extended this approach to observe the unsteady dynamics of a previously reported[10] three-parameter predator-prey DNA oscillator. Time-resolved fluorescence induced by prey was recorded to map the dynamic behaviour of each droplet, which allowed real-time monitoring of the decay or persistence of oscillations over three days. By generating a three-dimensional diagram, they identified the existence of rare, stochastically bursting oscillators near deterministic bifurcations. Such oscillators had been theoretically predicted but had not been experimentally observed. This finding highlights the potential of such microfluidic platforms to provide a panoramic view of the reaction landscape and to reveal the underlying mechanisms of complex biochemical reactions.

The droplet-based microfluidic platform can also be viewed as mimicking cells by providing program-controlled encapsulation which is similar to the compartmentalisation of biochemical reactions in living cells. The platform also operates like a microscale production line that can perform parameter optimization

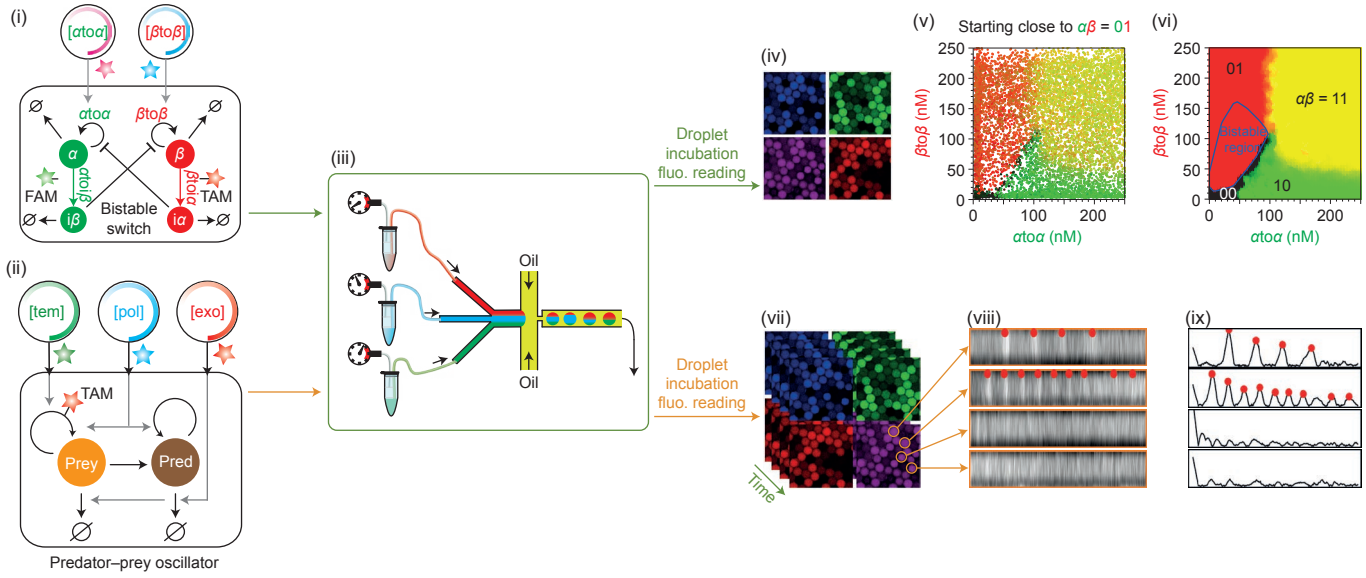

Figure 1 | High-resolution mapping bifurcation diagrams of nonlinear DNA circuits. (i) Circuit of a bistable DNA switch formed from two ssDNA triggers, α and β (reported separately by FAM, fluorescein and TAM, tetramethyl-6-carboxyrhodamine). (ii) Circuit of a predator–prey DNA oscillator. (iii) Schematics of the droplet-based microfluidic platform; the separate reaction components are mixed in a droplet encapsulated in oil. The fluorescence intensity of each channel is imaged by using confocal microscopy to measure either a single reading (iv) or a time-based series (vii). (v) Plotting the observables (α, β) against the parameters $(\alpha to \alpha, \beta to \beta)$ generates a scattered raw bifurcation diagram for the DNA switch. (vi) Smoothing of (v) generates the bifurcation diagram. (viii) Alternatively tracking the evolution of fluorescence in individual droplets yields kymographs that dynamically map the predator–prey oscillator. (ix) The time trace for each droplet is obtained by smoothing of the kymograph. Figure adapted from ref. 8, NPG.

in a highly parallel way with an integrated parallel readout. Furthermore, collective microscopic imaging of 10^4–10^6 droplets provides a high-resolution approach to mapping complex dynamics that are not limited to DNA reactions. In particular, enzymatic catalysis and protein signal transduction are far more complex and less predictable than DNA hybridization and this platform should also provide a quantitative tool for dissecting such biochemical systems with unprecedented precision. In addition to its demonstrated power in designing and optimizing biochemical circuits, this platform could inspire the engineering of commutations between droplets to construct tissue-like cooperating systems[11].

There are several key challenges to meet before this platform can find widespread application in solving fundamental and practical questions. First, the number of inlets in a microfluidic chip is still limited. This means that when the number of parameters increases, the platform may no longer be capable of monitoring all parameters simultaneously. Second, with higher dimensions, the number of data points must be increased to achieve the required resolution for mapping; however, the time required for generating the number of droplets required would also increase and so could become a limiting factor. Third, the precision of this droplet-based platform heavily relies on the speed, sensitivity and resolution of microscopes. Therefore, the development or adaptation of advanced microscopes remains a prerequisite. Nevertheless, after these challenges have been addressed, platforms based on the approach outlined by Rondelez and co-workers could be expected to nicely complement the computing power of DNA-based, or more generally, biomolecule-based computers, and to open up new opportunities for applying such networks to demanding real-world problems. ❐

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References

1. Hariadi, R. F. *et al. Nature Nanotech.* **10,** 696–700 (2015).
2. Douglas, S. M., Bachelet, I. & Church, G. M. *Science* **335,** 831–834 (2012).
3. Adleman, L. M. *Science* **266,** 1021–1024 (1994).
4. Benenson, Y. *Nature Rev. Genetics* **13,** 455–468 (2012).
5. Qian, L. & Winfree, E. *Science* **332,** 1196–1201 (2011).
6. Zhang, D. Y. & Winfree, E. *J. Am. Chem. Soc.* **131,** 17303–17314 (2009).
7. Hasatani, K. *et al. Chem. Commun.* **49,** 8090–8092 (2013).
8. Genot, A. J. *et al. Nature Chem.* **8,** 760–767 (2016).
9. Padirac, A., Fujii, T. & Rondelez, Y. *Proc. Natl Acad. Sci. USA* **109,** 4–6 (2012).
10. Fujii, T. & Rondelez, Y. *ACS Nano* **7,** 27–34 (2012).
11. Regot, S. *et al. Nature* **469,** 207–211 (2011).

WATER OXIDATION

Intermediate identification

The slow kinetics of light-driven water oxidation on haematite is an important factor limiting the material's efficiency. Now, an intermediate of the water-splitting reaction has been identified offering hope that the full mechanism will soon be resolved.

Alexander J. Cowan

The development of secure, sustainable low-carbon energy technologies is one of humanity's most urgent needs. Despite its abundance, solar energy remains vastly underutilized and to become our primary energy resource, its intermittency, both on the diurnal and seasonal timescales, need addressing. A highly promising solution is to use solar energy and abundant feedstocks such as water and carbon dioxide to produce fuels including hydrogen, methane and methanol in a sustainable manner. A common feature to the production of all of these solar fuels is the need to oxidize water.

Despite being studied for over 35 years, with considerable progress having been made[1], haematite (α-Fe_2O_3) remains only a promising material for light-driven water oxidation. Haematite meets many of the key criteria for application — it is cheap, stable and inherently scalable, consisting of only earth-abundant elements. However, the activity of haematite falls far below commonly cited 'solar to fuels' efficiency targets within the field (10%) and its own theoretical maximum (15%). Addressing the internal loss pathways of haematite is therefore potentially transformative to the field of solar fuels. In an exciting breakthrough described in *Nature Chemistry*, Zandi and Hamann provide the first structural assignment for an intermediate in the water-oxidation mechanism on a haematite surface[2], representing an important step towards the identification of the overall mechanism.

Understanding the mechanism of water oxidation is important because water splitting and O_2 evolution is a complex process, requiring four electron and proton transfers. Oxygen evolution is commonly cited as being the bottleneck in both natural- and artificial-light-driven water splitting. Previous spectroscopic[3] and electrochemical[4] measurements have shown that water oxidation is particularly slow on haematite and photogenerated positive charges (holes) persist for milliseconds-to-seconds at the haematite/water interface. This need to accumulate very long-lived holes provides a window of opportunity for electron–hole recombination to occur, lowering the material's internal efficiency. Although studies[5], including those using ultraviolet-to-visible absorption spectroscopy[3,4], identified the presence and timescales of water-splitting intermediates, the broad spectral features have prevented their definitive assignment to individual chemical states. Therefore the mechanism, and hence the reason for the slow surface kinetics, has remained experimentally unverified.

The successful identification of an intermediate at sub-monolayer concentrations on an operational photoelectrode surface in aqueous electrolyte is a remarkable achievement. Perhaps most surprising is that, to achieve it, Zandi and Hamann used a relatively well-established technique that is available in many laboratories worldwide. Through the application of attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, the authors are able to obtain sufficient sensitivity to gather information on a vibrational mode that was found to be present only during electrochemical and photoelectrochemical water splitting. ATR-FTIR spectroscopy has been used previously to explore photocatalytic water splitting[6,7], but this appears to be the first *in situ* study of a water-splitting photoelectrode.