

light microscopy. The authors also succeeded in loading the large Cas9 protein component of the gene-editing system CRISPR, and when these PVCs were supplied to cells with a guide RNA, specific gene editing (base editing) was detected.

Following on from their work on protein introduction to cultured cells *in vitro*, Kreitz and colleagues explored whether the re-engineered PVCs could function in a live animal. Purified PVC particles were injected directly into the hippocampus region of mouse brains. The authors observed the fluorescent signal of the cargo protein only around the injection area. Importantly for future biomedical applications, these PVC injections did not trigger local immune-cell activation and PVCs could no longer be detected seven days after the brain injection.

It was previously suggested that various bacterial secretion systems with the ability to inject molecules in a way that is dependent on cell–cell contact could serve as a tool for delivering cargo to target cells. By comparison, the use of extracellular contractile injection systems has two key advantages. First, their mechanism of conferring specificity is now well understood, which is not the case for bacterially anchored secretion systems. Second, extracellular contractile injection systems are defined assemblies that can be purified and applied to target organisms without the need for a treatment that requires live bacteria or minicells (small engineered artificial bacterial cells that lack chromosomes).

An interesting future direction would be to expand the target range of PVCs to include bacterial cells. Other studies have suggested that related extracellular contractile injection systems called pyocins could be used as antibacterial agents^{15,16}. Such efforts would widen the scope of future applications by providing a programmable platform that might overcome a potential issue in existing antibacterial phage therapy – namely, unwanted phage replication¹⁷.

Finally, the approaches developed by Kreitz and colleagues might be useful for studying and engineering other contractile injection systems. Despite their high diversity and multitude of functional roles, the systems characterized so far seem to rely on an evolutionarily conserved core set of components that have relatively minor structural differences that allow for notable mechanistic adaptations. This modular design might be harnessed in the future to try to develop, for example, engineered multi-barrelled complexes that form arrays of many individual contractile injection systems^{5,6}. These might enable more cargo to be delivered per target cell than with a single injection system.

The platform of PVCs, offering the reported customizable features of cargo and specificity, might be complemented by other platforms

and modules in efforts towards generating a powerful mix-and-match toolbox for research and medicine. Will these systems become commonplace in our labs and lives in the future?

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1. Kreitz, J. *et al. Nature* **616**, 357–364 (2023).
2. Brackmann, M., Nazarov, S., Wang, J. & Basler, M. *Trends Cell Biol.* **27**, 623–632 (2017).
3. Chen, L. *et al. Cell Rep.* **29**, 511–521 (2019).
4. Geller, A. M. *et al. Nature Commun.* **12**, 3743 (2021).
5. Böck, D. *et al. Science* **357**, 713–717 (2017).

6. Shikuma, N. J. *et al. Science* **343**, 529–533 (2014).
7. Jiang, F. *et al. Cell* **177**, 370–383 (2019).
8. Basler, M., Pilhofer, M., Henderson, G. P., Jensen, G. J. & Mekalanos, J. J. *Nature* **483**, 182–186 (2012).
9. Hurst, M. R. H., Beard, S. S., Jackson, T. A. & Jones, S. M. *FEMS Microbiol. Lett.* **270**, 42–48 (2007).
10. Yang, G., Dowling, A. J., Gerike, U., French-Constant, R. H. & Waterfield, N. R. *J. Bacteriol.* **188**, 2254–2261 (2006).
11. Xu, J. *et al. Nature Microbiol.* **7**, 397–410 (2022).
12. Hu, B., Margolin, W., Molineux, I. J. & Liu, J. *Proc. Natl Acad. Sci. USA* **112**, E4919–E4928 (2015).
13. Wang, X. *et al. Sci. China Life Sci.* **65**, 618–630 (2022).
14. Jiang, F. *et al. Sci. Adv.* **8**, eabm2343 (2022).
15. Scholl, D. *et al. Antimicrob. Agents Chemother.* **53**, 3074–3080 (2009).
16. Alqahtani, A. *et al. Can. J. Microbiol.* **67**, 919–932 (2021).
17. Klumpp, J., Dunne, M. & Loessner, M. J. *Curr. Opin. Microbiol.* **71**, 102240 (2023).

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Materials science

Excited states identified from molecular movies

Andrew J. Musser & Hannah Stern

A molecular process called singlet fission might boost solar-cell efficiency, but the mechanism must first be determined.

A technique that probes molecules undergoing this process finally reveals the excited states involved. **See p.275**

Over the past two decades, a strategy has emerged for turning waste heat produced by widely used solar cells into useful energy, based on a quantum-mechanical phenomenon called singlet fission¹. On page 275, Neef *et al.*² shed light on the steps involved in singlet fission in a molecular semiconductor called pentacene. The findings settle a long-standing debate about the sequence of events and bring us closer to using singlet fission to improve solar-cell efficiencies.

Sunlight is the most abundant source of sustainable and clean energy. However, to produce electricity at the scale needed by society, affordable solar cells are required that convert sunlight to electricity efficiently. Unfortunately, achieving highly efficient, low-cost solar cells is a major challenge, despite decades of effort. This is due to the apparent trade-off between the efficiency of solar cells and their cost and complexity.

For example, conventional ‘single junction’ silicon solar cells are cheap and simple, but have a fundamental efficiency cap of about 30% (ref. 3). This limit arises because these solar cells are most efficient at converting photons (single particles of light) of a particular energy to electricity, whereas sunlight has a broad energy spectrum. The lower-energy photons in sunlight are not absorbed by

silicon, whereas higher-energy ones generate unwanted heat.

So how could singlet fission help? Singlet fission occurs in molecular semiconductors, and describes the splitting of one ‘bright’ light-induced excitation (a singlet) into two ‘dark’ excitations of lower energy, called triplets. This process produces two excited electrons from just one photon, and has the potential to greatly increase the output of electric current and the efficiencies of conventional solar cells. The phenomenon of singlet fission was first described⁴ in 1965, but it was not until 2004 that the link to solar cells was made⁵. However, its promise in this regard has yet to be realized, with only a few reports showing that the efficiency of silicon solar cells can be enhanced by this phenomenon (see ref. 6, for example).

The main challenge has been developing molecules capable of singlet fission that are suitable for use in silicon solar cells, with a key bottleneck being the limited understanding of the underlying process. Despite decades of research, and widespread agreement that fission does indeed occur and can be amazingly fast (on subpicosecond timescales; 1 ps is 10⁻¹² seconds), the fundamental mechanism is still debated. Among the possible phenomena to be implicated so far are quantum-coherent

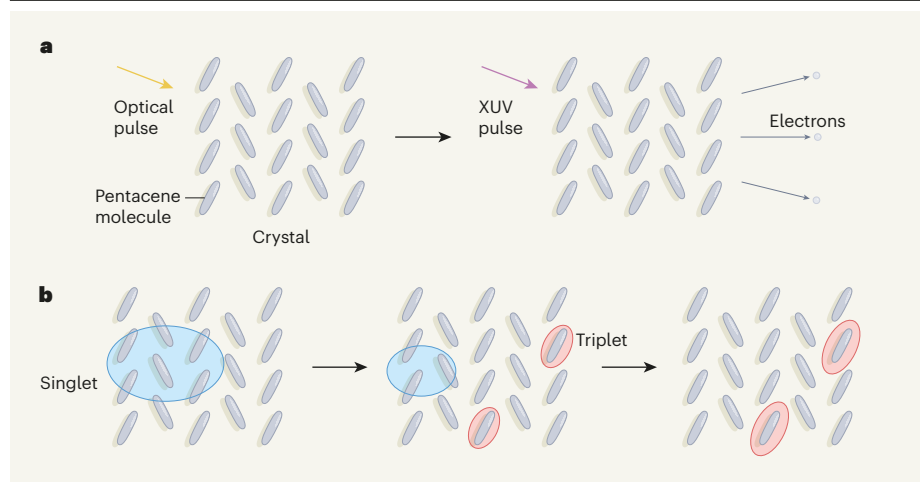


Figure 1 | Observing excited states in singlet fission. The phenomenon of singlet fission occurs in molecular semiconductors, and could be used to increase the efficiency and electrical output of conventional solar cells. In this process, a light-induced excitation (a singlet) is split into two excitations of lower energy (triplets). **a**, Neef *et al.*² used an optical pulse to excite molecules in crystals of the semiconductor pentacene, triggering singlet fission. A second pulse, of extreme ultraviolet (XUV) light, stimulates the emission of electrons, which are analysed to identify the energy and momentum of the excited states. **b**, By analysing a time series of ‘snapshots’ of the electrons, the authors observed that the initial singlet state is delocalized over several pentacene molecules, but then transitions into triplet states localized around specific molecules. The findings allowed the singlets to be categorized as a type of excitation known as a charge-transfer state.

interactions⁷ (which involve the formation of a superposition of bright and dark states directly after light absorption); charge-transfer processes that result in electrons being shared across multiple molecules⁸; and strong coupling of electronic excitations to the motions of molecules as they jiggle about after excitation⁹. The principles for designing better molecules for singlet fission would be different for each of these mechanisms, so distinguishing between them is clearly important. But working out which mechanism occurs has been difficult – until now.

Previously, the best approach to probing the evolution of short-lived excitations in materials was to record a ‘molecular movie’: capturing a series of snapshots of how molecules absorb light over time using extremely fast ‘shutter speeds’ (much shorter than 1 ps)¹⁰. The various excited states produced in such experiments each generate their own energetic fingerprints, but sometimes with closely related features. The great difficulty has been reliably assigning such features to the correct state.

Neef *et al.* take a different approach by developing a new technique for the field: time- and angle-resolved photoemission spectroscopy (trARPES; Fig. 1). Similarly to previous methods, trARPES uses a precisely synchronized series of laser pulses to stimulate the ejection of electrons from molecules in materials; the evolving energetic fingerprints of excited molecules can then be probed by analysing these electrons. The crucial difference from previous methods is that the detector records electron momentum, as well as energy. This seemingly small change provides

invaluable and previously inaccessible information about the shape of excited electron clouds, and how the clouds spread between molecules. It is as if molecular movies, having previously been recorded in black and white, now suddenly appear in full colour.

Using trARPES, Neef *et al.* obtained a complex 4D data set that they were able to decompose to obtain the signature orbital profiles – the shapes of the electron clouds – of every excitation formed during the singlet fission process. This analysis revealed that the initial bright state is delocalized across several molecules and has multiple orbital signatures. That is, it is best described as a mixture of several kinds of electronic excitation.

One of the mixture’s components is the long-sought charge-transfer state, which is what enables the excitation to spread between molecules. The authors observed that, during fission, these collective singlet signatures decayed over about 100 femtoseconds (1 fs is 10⁻¹⁵ seconds) to form dark triplets that are highly localized to individual molecules. The findings can be described by a simple kinetic scheme that rules out an earlier proposal of a quantum coherent pathway⁷, and instead strongly supports the idea that charge-transfer states form as the first step during singlet fission⁸.

This result helps to unify various strands of singlet-fission research from the past decade. However, it remains to be seen how well the picture developed by Neef *et al.* for pentacene and similar compounds applies to chemically different, more practically useful materials. The same basic phenomena – rapid

conversion between bright and dark states, driven by mixing between different types of excitation – are thought to occur in a host of other processes in molecular materials (see refs 11, 12, for example). As long as stable crystals of such materials can be prepared, then Neef and colleagues’ powerful method for picking out the fingerprints of excitations promises to shed light on the mechanisms involved, and on how to use these materials for applications ranging from energy-efficient lighting to quantum-information science.

The authors’ orbital-resolved method provides the most intimate picture yet of what happens during singlet fission. But it is just as blind as earlier techniques to how the excited states transition between each other – the key issue when it comes to designing improved molecular materials for potential applications. So, although Neef and colleagues’ ‘colour’ molecular movies provide invaluable insight beyond what could be gleaned from their ‘black-and-white’ predecessors, more work is needed to know what’s happening between the frames.

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1. Smith, M. B. & Michl, J. *Chem. Rev.* **110**, 6891–6936 (2010).
2. Neef, A. *et al. Nature* **616**, 275–279 (2023).
3. Shockley, W. & Queisser, H. J. *J. Appl. Phys.* **32**, 510–519 (1961).
4. Singh, S., Jones, W. J., Siebrand, W., Stoicheff, B. P. & Schneider, W. G. *J. Chem. Phys.* **42**, 330–342 (1965).
5. Nozik, A. J. *et al. Proc. 27th DOE Solar Photochem. Res. Conf.* 63–66 (US Dept Energy, 2004).
6. Einzinger, M. *et al. Nature* **571**, 90–94 (2019).
7. Chan, W.-L. *et al. Acc. Chem. Res.* **46**, 1321–1329 (2013).
8. Berkelbach, T. C., Hybertsen, M. S. & Reichman, D. R. *J. Chem. Phys.* **138**, 114103 (2013).
9. Bakulin, A. A. *et al. Nature Chem.* **8**, 16–23 (2016).
10. Wilson, M. W., Rao, A., Ehrler, B. & Friend, R. H. *Acc. Chem. Res.* **46**, 1330–1338 (2013).
11. Scholes, G. D. *et al. Nature* **543**, 647–656 (2017).
12. Penfold, T. J., Gindensperger, E., Daniel, C. & Marian, C. M. *Chem. Rev.* **118**, 6975–7025 (2018).

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