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lack of nonthermal x-ray and TeV γ -ray emission in the northwestern rim of the remnant indicates that, if there is a cosmic ray precursor, the injection of electrons is little and the particle energies are low (<1 MeV), but cosmic ray acceleration can still happen.

Our pilot project demonstrates the feasibility of using integral-field spectroscopy to observe and study the microphysics of high-velocity shocks around supernova remnants. The resulting high spatial resolution mapping of the Balmerdominated shocks in the northwestern rim of SN 1006 suggests the presence of suprathermal protons of energies 10 to 100 keV, which can seed high-energy cosmic rays.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1228297/DC1 Materials and Methods Supplementary Text Figs. S1 to S3 Table S1 References (*30–33*) 1 August 2012; accepted 7 February 2013 Published online 14 February 2013; 10.1126/science.1228297

A Tissue-Like Printed Material

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Living cells communicate and cooperate to produce the emergent properties of tissues. Synthetic mimics of cells, such as liposomes, are typically incapable of cooperation and therefore cannot readily display sophisticated collective behavior. We printed tens of thousands of picoliter aqueous droplets that become joined by single lipid bilayers to form a cohesive material with cooperating compartments. Three-dimensional structures can be built with heterologous droplets in software-defined arrangements. The droplet networks can be functionalized with membrane proteins; for example, to allow rapid electrical communication along a specific path. The networks can also be programmed by osmolarity gradients to fold into otherwise unattainable designed structures. Printed droplet networks might be interfaced with tissues, used as tissue engineering substrates, or developed as mimics of living tissue.

C ells in living tissues communicate with each other in a controlled manner. As a consequence, tissues display the emergent properties that distinguish them from collections of independently functioning cells. The reproduction of similarly sophisticated behavior in a synthetic system would be of scientific and technological value but is not feasible with existing artificial membrane platforms. Of the various classes of structures containing artificial bilayers, liposomes resemble cells most closely, but the controlled insertion of specialized membrane proteins such as gap junctions (1) would be required to join liposomes and form a cohesive, cooperative system.

Lipid-coated aqueous droplets in oil adhere at their interfaces to form stable bilayers (2–5), which can be functionalized with membrane proteins.

Small two-dimensional (2D) networks of droplets connected in this way have been shown to act cooperatively as light sensors (5), batteries (5), or simple electrical circuits (6). Further, the droplets can release their contents to bulk aqueous solution after a change in pH or temperature (7-9). Droplet networks are therefore a promising platform for the construction of complex functional devices. However, functional networks have been limited to small groups of droplets assembled by manual (5-7) or mechanical (10) manipulation. microfluidic means (3, 11, 12), or external fields (13-15). Larger assemblies have been constructed by packing droplets into microfluidic containers (16, 17), but their complexity is limited by the uncontrolled filling process.

We automatically printed tens of thousands of heterologous picoliter droplets in softwaredefined, 3D millimeter-scale geometries. The resulting macroscopic material is cohesive and self-supporting and consists of distinct aqueous compartments partitioned by single lipid bilayers. Printing can take place in bulk oil or within oil

drops that reside in aqueous solution. The bilayers can be functionalized with membrane proteins to allow electrical communication along a specific route. Printed droplet networks can also be programmed by osmolarity gradients to fold after printing into various designed geometries not accessible by direct printing. Three characteristics distinguish printed networks from other shape-changing materials such as the bimetallic strip or hydrogels patterned to undergo nonuniform volume changes under external stimuli (18, 19): droplet networks are readily printed, they consist of compartments that can communicate through membrane proteins, and their folding is driven by internal differences in osmolarity. The latter characteristics make the folding behavior of droplet networks closely analogous to the nastic movements exhibited by certain plants (20, 21). Printed picoliter droplet networks constitute a defined synthetic platform for sophisticated collective behaviors (Fig. 1A) and might be developed for medical applications (22).

Our strategy was to eject aqueous droplets (fig. S1 and supplementary text S1) within a bath of lipid-containing oil that was mounted on a motorized micromanipulator, so that a droplet network was built up in horizontal lavers (Fig. 1B). The construction of droplet networks raises challenges that preclude the use of a commercially available printer (supplementary text S2), which we addressed with a specially designed system (23) (figs. S2 to S8 and table S1). A network is defined in horizontal cross sections one droplet thick (Fig. 1C), and a custom computer program (23) accordingly synchronizes the motion of the oil bath with the ejection of droplets from two droplet generators to construct the desired network (Fig. 1D). We have printed precisely defined networks consisting of up to

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Fig. 1. Printed droplet networks. (A) Illustration of a printed droplet network. **(B)** Schematic of the printing process. Two droplet generators eject droplets, typically ~65 pl, of different aqueous solutions into a solution of lipids in oil. The oil bath is mounted on a motorized micromanipulator. The droplets acquire a lipid monolayer and form bilayers with droplets in the growing network. **(C)** Horizontal cross-sections of a design for a 3D droplet

network with a branching structure (blue) embedded in a cuboid (gray). The design comprises 20 layers of 50×35 droplets each; only alternate layers are shown. (**D**) Network printed according to the design in (C). Scale bar, 5 mm. (**E**) Schematic of a 3D design that consists of 28 layers of 24×24 droplets each. (**F**) Three orthogonal views of a single network printed according to the design in (E). Scale bar, 1 mm.



Fig. 2. Droplet networks printed in bulk aqueous solution. (A) Schematic of printing in aqueous solution. Aqueous droplets are ejected into a drop of oil suspended in bulk aqueous solution. Excess oil can be removed after printing by suction through a printing nozzle. (B) Micrograph of a network printed in aqueous solution, viewed from above. A core of orange droplets is surrounded by a shell of blue droplets, which contain the

fluorescent dye pyranine. Scale bar, 400 μ m. (**C**) Horizontal sections of the network in (B) obtained by confocal microscopy, showing the fluorescent shell of droplets around the nonfluorescent core. The sections span approximately the bottom 150 μ m of the network. Scale bar, 400 μ m. (**D**) Micrographs of three other networks printed in bulk aqueous solution. Scale bars, 400 μ m.

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~35,000 heterologous droplets ejected at a rate of ~1 s⁻¹ (Fig. 1, C to F).

Printed droplet networks are self-supporting (Fig. 1, D and F), and a thermodynamic analysis of the system indicates that stable networks can be printed with at least several thousand layers (23). The lattice of lipid bilayers also allows droplet networks to retain their shape under gentle perturbation; each bilayer lends an effective spring constant of ~4 mN m⁻¹ to connected droplets, with a tensile strength of ~25 Pa (23) (figs. S9 and S10). We estimate that Young's modulus of printed networks is on the order of ~100 to 200 Pa (23) (fig. S11), which is comparable to the elastic moduli of brain, fat, and other soft tissues (24).

We have shown (7) that droplet networks can be stabilized in bulk aqueous solution by encapsulation within small drops of oil, for prospective applications in synthetic biology and medicine. Whereas those networks were created manually and therefore were limited in complexity, in the present experiments, we demonstrated the printing of encapsulated networks consisting of thousands of droplets in designed 3D patterns. This was achieved by printing inside an oil drop suspended in aqueous solution (23) (Fig. 2A and supplementary text S3). Once printing is complete, excess oil can be removed by suction through one of the printing nozzles. Encapsulated printed networks (Fig. 2, B to D) were stable for at least several weeks and will therefore serve to expand the functions previously demonstrated with simple encapsulated networks, including communication with the aqueous surroundings through membrane pores, and pH- or temperature-triggered release of contents (7).

To establish whether membrane proteins could be included in specific bilayers, we printed a network in which only the droplets along a defined pathway contained staphylococcal α -hemolysin (α HL), in order to create an ionically conductive route across an otherwise insulating network (Fig. 3, A and B). To probe the network electrically, a drop of buffer of ~500 µm in diameter containing aHL pores was manually pipetted onto each of two Ag/AgCl electrodes with agarose-coated tips. The drops were then brought into contact with different parts of the network, so that they formed bilayers with the droplets on the network surface (Fig. 3A). When the two large drops were placed on either end of the α HL-containing pathway (Fig. 3B), we measured a stepwise increase in ionic current under an applied potential (Fig. 3C). After one of the drops was separated and brought back into contact with the network away from the αHL-containing pathway (Fig. 3D), only transient currents were observed (Fig. 3E). When this drop was separated from the network again

and replaced in its original position, a stepwise increase in current was again observed (fig. S12). Droplet networks in which no droplets contained α HL showed negligible current flow, whereas the current measured across droplet networks in which every droplet contained α HL was similar to that shown in Fig. 3C (fig. S12).

To interpret these results, we performed computational simulations of the electrical behavior of printed droplet networks (23) (fig. S13). The electrical model was consistent with the measured currents exemplified in Fig. 3, C and E, provided that most of the bilayers along the aHL-containing pathway contained several pores and the other bilayers in the network contained none (supplementary text S4 and fig. S14), so that the pathway presented an already established conductive route through the otherwise insulating network. The stepwise increase in current in Fig. 3C was most likely caused by pore insertions into the bilayers between the large electrode drops and the pathway droplets, rather than into bilayers within the printed network (supplementary text S4). The current spikes in Fig. 3E correspond to pore insertions into the bilayers between the electrode drop newly placed away from the pathway and insulating droplets in the network, which transmit transient capacitive currents but do not permit a steady resistive current (supplementary text S4). Based on these findings,



Fig. 3. Electrically conductive pathway. (A) Schematic of part of a network printed with an ionically conductive pathway. Only the green droplets and the large drop contain α HL pores. The large drop is impaled with an Ag/AgCl electrode. The magnified section illustrates the α HL pores in the bilayers around the α HL-containing droplets. (B) Photograph of a printed network with electrode-impaled drops placed on either end of the conductive pathway. The green droplets contain α HL, whereas the other

droplets contain no protein. Scale bar, 500 μ m. (**C**) Stepwise increase in the ionic current as measured in the configuration in (B), at 50 mV in 1 M KCl at pH 8.0. (**D**) Photograph of the network in (B), after separating one of the large drops and rejoining it onto the network away from the conductive pathway. Scale bar, 500 μ m. (**E**) Selected portions of a single recording as measured in the configuration in (D) at 50 mV, showing transient increases in ionic current.

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we maintain that droplet networks can be printed with protein pores in specific bilayers. The tolerance of printed networks to slight variations in structure and in the number of proteins that insert in each bilayer may be compared to the robustness of living tissues to minor flaws. The printed network presented here is functionally analogous to a nerve axon in enabling rapid, long-distance electrical communication along a defined path, but it does not mimic an axon's mechanism of signal propagation.

Finally, we explored a means for droplets in a printed network to produce a designed macroscopic change in the network geometry through cooperative action. Water permeates readily through droplet interface bilayers even in the absence of protein channels or pores, with a permeability coefficient of 27 \pm 5 $\mu m~s^{-1}$ (mean \pm SD, n = 3 pairs of droplets joined by bilayers) under the conditions of this study (fig. S15), consistent with other permeability measurements of droplet interface bilayers (25-27) and other lipid bilayer systems (28). Consequently, two droplets of higher and lower osmolarity joined by an interface bilayer will respectively swell and shrink until their osmolarities are equal (Fig. 4A). By extension, water transfer within a network composed of droplets of different osmolarities will cause spontaneous deformation of the network as long as adhesion between droplets is maintained (Fig. 4B).

We found several prerequisites for droplet networks to fold in a predictable way (see also supplementary text S5). First, to prevent droplets from being printed onto incorrect positions in the network, the network must fold slowly as compared to the printing time. Second, the swelling and shrinking of the two droplet types induces a stress between regions of connected droplets that can cause the network to buckle in an uncontrolled manner. This is analogous to the buckling instability in tissues that grow at inhomogeneous rates, such as certain leaves (29) and flower petals (30). In certain cases, the stress may instead cause connected droplets to separate and thereby prevent the network from folding further around the fracture zone (fig. S16). These various problems can be solved through judicious choices of printing rate, salt concentrations, and droplet size, and adjustments to the network geometry (supplementary text S5). The final geometry of the network is then determined in a well-defined way by its initial geometry, the distribution of the two types of droplets, and the ratio of their osmolarities. Using a simple computational model that allows us to predict the folding behavior of a given droplet network (23) (table S2), we designed droplet networks that folded successfully.

In one experiment, we printed a network that comprised two strips of droplets of different salt concentrations, connected along their lengths

(Fig. 4C). The network folded spontaneously in the horizontal plane over ~3 hours, until droplets at opposing ends of the network formed new bilayers in a closed ring. We also programmed a network to fold spontaneously out of the horizontal plane to attain a geometry that would be difficult to print directly. We printed a flowershaped network with four petals, in which the lower layers had a higher osmolarity than the upper layers. The permeation of water from the upper into the lower layers induced a curvature that raised the petals and folded them inward (Fig. 4D and movie S1). The folded network was self-supporting and approximated the geometry of a hollow sphere, with the originally upper layer contained within a shell formed by the originally lower layer (Fig. 4E and fig. S17). The evolution of the geometry of the network is in good agreement with that of a simulated folding network with similar initial conditions (Fig. 4E and movie S2). We estimate the energy available from the osmosis-driven flow of water to be on the order of $1 \mu J$ for this network (23), which is comparable to the total energy of bilayer formation in the network and is several orders of magnitude greater than the energy required to lift the droplets against gravity (23).

We have devised a means of printing, in designed 3D geometries, a soft material composed of aqueous microcompartments bound together by lipid bilayers. The bilayers allow the



Fig. 4. Self-folding droplet networks. (**A**) Schematic of two droplets of different osmolarities joined by a lipid bilayer. The flow of water through the bilayer causes the droplets to swell or shrink. (**B**) Schematic of a droplet network that comprises two strips of droplets of different osmolarities. The transfer of water between the droplets induces an overall deformation of the network. (**C**) Photographs of a rectangular network folding into a circle over ~3 hours. The orange and blue droplets initially contained 250 mM KCL

and 16 mM KCl, respectively. Scale bar, 250 μ m. (**D**) Photographs of a flowershaped network folding spontaneously into a hollow sphere. The orange and blue droplets initially contained 80 mM KCl and 8 mM KCl, respectively. The photographs cover a period of 8 hours. Scale bar, 200 μ m. (**E**) Frames from a folding simulation of a network with a similar initial geometry to the network in (D). Blue and red represent the lowest and highest initial osmolarities, respectively, and white indicates the average of the two.

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compartments to interact directly through membrane proteins or osmotic flows of water and thereby enable the engineering of collective properties such as long-range electrical communication or macroscopic deformation. We used the well-characterized aHL pore and simple salt solutions to achieve cooperative action between droplets in a printed droplet network. Additional membrane proteins and their engineered forms (5, 6) should allow printed networks to transduce a wider range of signals, and stimulus-responsive osmolytes might offer greater control of folding. An interesting challenge is the integration of droplet networks with living organisms. The outer surface of a printed network might be engineered to interact in a designed way with a physiological environment; for example, to deliver drugs upon a specific signal (7-9). More sophisticated networks might be interfaced with failing tissues to support their functions. Alternatively, cells could be included inside the droplets during printing for various applications, such as to immobilize cells within a defined 3D dimensional scaffold for tissue engineering (22).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6128/48/DC1 Materials and Methods Supplementary Text S1 to S5 Figs. S1 to S17 Tables S1 and S2 References (31-36) Movies S1 and S2

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Broadband 2D Electronic Spectroscopy Reveals a Carotenoid Dark State in Purple Bacteria

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Although the energy transfer processes in natural light-harvesting systems have been intensively studied for the past 60 years, certain details of the underlying mechanisms remain controversial. We performed broadband two-dimensional (2D) electronic spectroscopy measurements on light-harvesting proteins from purple bacteria and isolated carotenoids in order to characterize in more detail the excited-state manifold of carotenoids, which channel energy to bacteriochlorophyll molecules. The data revealed a well-resolved signal consistent with a previously postulated carotenoid dark state, the presence of which was confirmed by global kinetic analysis. The results point to this state's role in mediating energy flow from carotenoid to bacteriochlorophyll.

arotenoids (Cars) play an important role in the photoreactions of photosynthesis (1). Being accessory pigments, they absorb light in the spectral region not accessible to chlorophylls (Chls) and, by subsequent energy transfer to Chls, they increase the spectral crosssection for photosynthetic activity. Moreover, in

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some organisms, most of the light that drives photosynthesis is absorbed by Cars (2). However, in spite of many years of studies, the exact mechanism of energy transfer from Cars to Chls remains controversial (3, 4), owing to the complex electronic structure of Cars.

Traditionally, Car photophysics is described by a three-level model, in which the ground stateto- $S_1(2A_g^{-})$ state transition is symmetry-forbidden and the light is absorbed by the $S_2(1B_u^+)$ state. After excitation, the S2 state rapidly decays in a few hundred femtoseconds to the "dark" S1 state, which then decays back to the ground state (S_0) or lA_g^{-}) on a picosecond time scale. However, the picture becomes more complicated for Cars with nine or more conjugated double bonds, for which Tavan and Schulten predicted the appearance of other dark states between the S₂ and S₁ states (5), in particular, the $1B_{\mu}^{-}$ state. In 2002 Cerullo et al. (6), using sub-10-fs pulses to excite isolated Cars, reported the presence of a kinetic intermediate phase (called S_x) that was populated by decay of the S2 state and then decayed into the S1 state. However, the interpretation of this phase as the dark electronic state has been controversially discussed in later studies (7, 8). Moreover, it remained unclear whether such an intermediate electronic state is actually involved in Car-to-Chl energy transfer.

In this work we investigated how the complex manifold of Car electronic states in the peripheral light-harvesting complex LH2 from the purple bacteria Rhodopseudomonas acidophila strain 10050 and Rhodobacter sphaeroides strain 2.4.1 couple to the Q_x state of bacteriochlorophyll (BChl). By comparing the 2D spectra of LH2 complexes with spectra of isolated Cars, we deduce the presence of a Car dark state in the vicinity of the Q_x state. A global kinetic analysis suggests several energy transfer pathways associated with this dark state. The results demand reexamination of the intermolecular energy transfer mechanisms in light-harvesting proteins.

The molecular structure of the LH2 complex from Rps. acidophila and corresponding ladder of

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