other subtly distinct phases segregating in the sample. It is now understood that it is a particular ordered phase of oxygen interstitials — called the Q2 phase from its diffraction signature — that hosts the highest temperature superconductivity. A typical sample will consist of ordered Q2 crystals of submicrometre size embedded in a glassy matrix where the interstitials are disordered. Optimal superconductivity occurs when conditions are such that the Q2 phase percolates into a fractal network, as shown by a previous paper by the same group[3].

That the existence of a robust superconducting phase depends on oxygen order rather than just the average hole doping is in itself interesting, though not unprecedented. But this now becomes a route to control, by making use of the high mobility of oxygen ions in this solid. Above 370 K the Q2 phase becomes disordered, so that if a sample is heated above this temperature and then quenched to low temperature, it has a poor superconducting order. However, by illuminating such a disordered sample with X-rays (Fig. 1), Poccia *et al.* observed nucleation and growth of the ordered domains, and a recovery of a robust high T_c state. The timescale for the growth with X-ray intensity makes it clear that X-ray illumination is the source — indeed photo-switching of phase transitions is known elsewhere in oxides. As an interesting sidebar, the growth shows an unexpected behaviour with intensity and time, accelerating when it has reached a threshold, implying that cooperative effects couple the domains as they grow, possibly a strain-mediated funnel to the ordered state.

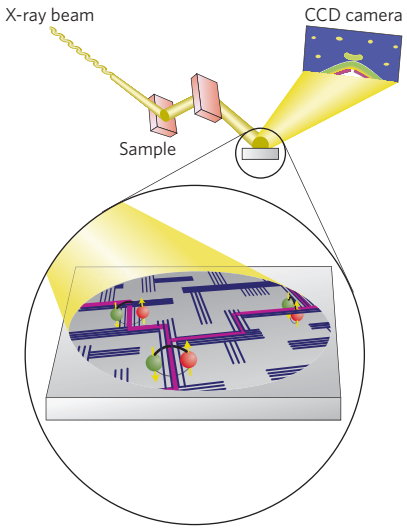

Figure 1 | Scheme of the set-up used by Poccia *et al.* to write and monitor robust superconducting regions in a disordered matrix[1]. The X-ray beam is focused on the sample and then a charge-coupled device (CCD) camera is used to detect the X-ray reflection.

In a final flourish, Poccia *et al.*[1] demonstrate the writing of the ordered Q2 phase with a dot and line on the scale of 100 micrometres. Although this is certainly not yet nanotechnology, there seems to be no reason in principle why this could not be extended down to modern submicrometre lithographic scales with the appropriate tools.

The cuprates — as exemplars of transition metal oxides in general — continue to demonstrate their multiphase complexity (structural, magnetic and electronic). It is this complexity that has hindered physicists' search for the 'mechanism' of high T_c. However, once tamed, this complexity will provide many avenues to control functionalities. Oxygen defects — both interstitials and vacancies — may prove to be one of the most beneficial. Modern techniques for atomically precise growth of oxide films and heterostructures have strongly implicated oxygen defects as controlling other electronic states[4], including magnetism and ferroelectricity. These methods allow precise doping on the nanoscale, allowing one to contemplate modulation doping of oxide electronics (paralleling conventional semiconductor technology) but with much greater functionality: not only superconducting electronics but possibly even processes such as electronic control of redox chemistry with new applications to batteries, photovoltaics and catalysis[5].

Having struggled to get to the source of the complexity in such materials and its origin on the atomic scale, we can be sure that though worthwhile, the road will not be easy. There will be many more hopeful commentaries to follow this one. ❐

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References

1. Poccia, N. *et al. Nature Mater.* **10,** 733–736 (2011).
2. Lee, P. A., Nagaosa, N. & Wen, X-G., *Rev. Mod. Phys.* **78,** 17–85 (2006).
3. Fratini, M. *et al. Nature* **466,** 841–844 (2010).
4. Brinkman, A. *et al. Nature Mater.* **6,** 493–496 (2007).
5. Bristowe, N. C., Littlewood, P. B. & Artacho, E. *Phys. Rev. B* **83,** 205405 (2011).

PROTEIN-PATTERNED HYDROGELS

Customized cell microenvironments

Mimicking the complexity of the extracellular environment in synthetic hydrogels is hard. A simple two-photon excitation strategy to simultaneously immobilize multiple proteins with spatial control in three dimensions shows promise.

Jennifer L. West

In tissues, cells generally grow embedded in the extracellular matrix (ECM) — a cross-linked hydrogel that includes polysaccharides as well as structural, signalling and cell-adhesive proteins. The local composition and structure of the ECM governs the biochemical and biomechanical environments experienced by individual cells, as receptors on their surface interact with distinct ECM components (Fig. 1a). Signals from cell–ECM interactions, along with certain cell–cell interactions, are integrated intracellularly to influence cell fate. This remarkably complex environment in which cells *in vivo* live makes it hard to recapitulate aspects of physiology *in vitro* to optimize cell growth, control cellular functions, or even mimic disease states. Although synthetic hydrogels with bioactive ligands that mimic key aspects of the ECM microenvironment have

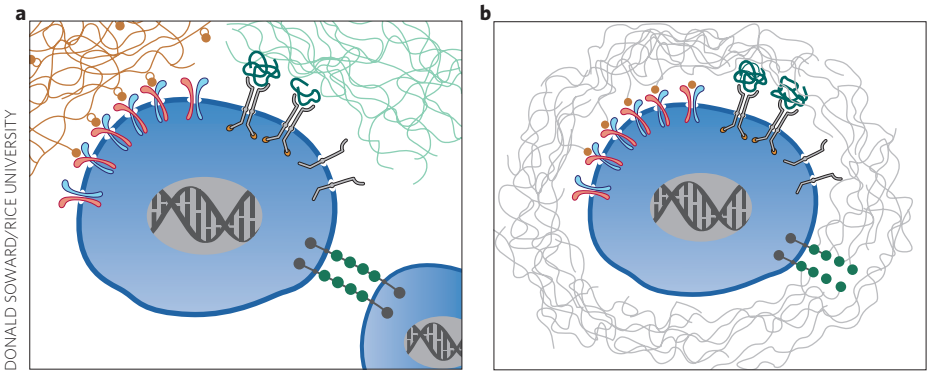

Figure 1 | Mimicking the extracellular environment in synthetic hydrogels. **a**, Cells interact with multiple components of the extracellular matrix, often in spatially segregated domains. Localized variations in ECM composition and organization can have profound impacts on cell behaviour, for example in defining stem-cell niches within a tissue otherwise composed of differentiated cells[10]. **b**, Cell–matrix and cell–cell interactions can be recapitulated in biomimetic hydrogels, and 3D micropatterning technologies can allow spatial control over the presentation of bioactive ligands. Spatial patterning in the ECM can reach subcellular scales, with localization of certain types of ligand within distinct patches and differences in cellular responses based on the areas of the patches[11].

been developed, technologies to spatially micropattern proteins in hydrogels in three dimensions are only now starting to emerge. Writing in *Nature Materials*, Wylie *et al.* demonstrate the simultaneous immobilization of multiple proteins in hydrogels with three-dimensional (3D) spatial control[1]. The method should advance our ability to control cellular interactions with materials-based approaches.

In most cell-culture applications, cells are grown in polystyrene dishes and interact with proteins that have adsorbed to the dish's surface. However, two-dimensional cell-culture environments have significant limitations, such as the intrinsic differences in cellular interactions with respect to 3D environments, the rigidity of the materials used for cell culture and the low degree of control over cell–adhesion interactions and signalling. Moreover, the growth of cells in 3D gels made of collagen or reconstituted ECM proteins has recently shown that cellular responses in such systems are often dramatically different from observations made on polystyrene surfaces, and that in general they more closely reflect tissue physiology[2].

The general premise for the design of ECM-mimicking 3D materials has been to employ a bioinert base material — such as poly(ethylene glycol), poly(vinyl alcohol) or agarose — which is then modified with appropriate cell-adhesive or cell-signalling sequences. Ideally, these material platforms can be designed in a modular manner to facilitate the fabrication of customized cell matrices for different experiments or applications. Some of the biomimetic modifications that have been made to synthetic hydrogels to mimic key cell–cell interactions include immobilization of cell-adhesive peptides[3], growth factors[4] and the membrane-attached receptors ephrins[5]. Synthetic hydrogels have also been rendered susceptible to degradation by their modification with cellular proteases involved in migration and ECM

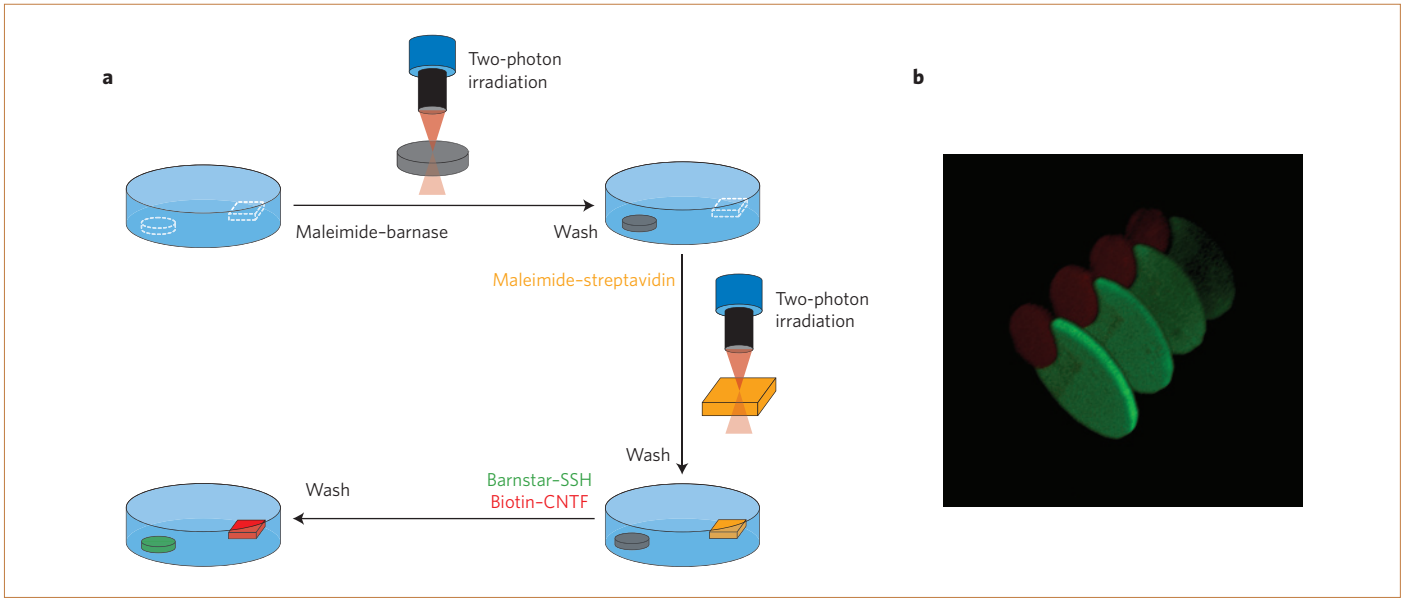

Figure 2 | Simultaneous immobilization of multiple proteins in 3D hydrogels. **a**, Sketch of the two-photon photochemical method used by Wylie *et al.*[1] to pattern SSH (green circle) and CNTF (red square). Thiol-reactive maleimide-modified barnase (black circle) and streptavidin (orange square) binding proteins are sequentially immobilized in the irradiated volume of the thiol-modified hydrogel (blue). After soaking the fusion proteins barstar–SSH and biotin–CNTF in the hydrogel, the proteins simultaneously bind to their respective partners (barnase–barstar and streptavidin–biotin) in the respective irradiated volumes. Intermediate washing steps in buffer are necessary to remove unbound proteins. **b**, Micrograph of the stack of layers below the surface of the hydrogel (400, 500, 600 and 700 μm from left to right) patterned with SSH (green) and CNTF (red) in an eye-like shape[1]. The amount of protein immobilized is indicated by the colour intensity. The resolution is 5 μm in the layer plane and 40 μm across the layers (*z* direction). Resolution in the *z* direction is always poorer because of the shape of the excitation volume under two-photon irradiation[9], although previous work with two-photon excitation of photoinitiators to induce cross-linking of acrylate-terminated polymers has achieved resolution to at least 5 μm in the *z* direction when illumination conditions were appropriately optimized[8].

remodelling[6], mimicking the dominant mode of material biodegradation in the ECM.

Among the emerging 3D patterning technologies that can be applied to biomimetic hydrogels to allow spatially controlled presentation of multiple bioactive ligands (Fig. 1b), two-photon excitation strategies show the greatest promise because they allow the fabrication of complex, free-form shapes with micrometre-scale resolution. This can be achieved because two-photon excitation events can be limited to a very small focal volume, and the microscope objective through which the focusing of the laser beam takes place[7] can be moved relative to the sample to spatially control excitation and thus chemical modification (Fig. 2a). This can be done iteratively for the immobilization of multiple species[1,8]. The technique has been applied to control the immobilization of bioactive peptides in biomimetic hydrogels, to locally alter mechanical properties, to generate gradients of immobilized factors and to fabricate complex pore geometries. Interestingly, two-photon approaches have been used to photodegrade components in hydrogel materials[9].

By using two-photon chemistry, Wylie and colleagues[1] show the feasibility of patterning two proteins — the stem-cell differentiation factors sonic hedgehog (SHH) and ciliary neurotrophic factor (CNTF) — as opposed to the shorter bioactive peptides that have been previously immobilized in two-photon patterning studies. The authors' chemical immobilization approach is based on the iterative uncaging of thiol groups on two-photon irradiation, followed by reaction with barnase and streptavidin proteins modified with thiol-reactive maleimide compounds. The thiols act as anchoring sites for the simultaneous immobilization of the two binding proteins, which subsequently form complexes with the fusion proteins barstar–SSH and biotin–CNTF (Fig. 2a). The use of the orthogonal binding pairs barnase–barstar and streptavidin–biotin should be applicable to a broad range of proteins (provided they can be biotinylated or made as barstar fusion proteins), thus expanding the options available for hydrogel modification. With this technique Wylie and co-authors fabricated stacks of patterned agarose hydrogel layers (Fig. 2b), and show that the pair of fusion proteins barstar–SSH and biotin–CNTF remained bioactive after immobilization: retinal progenitor cells cultured on the surface of the protein-patterned hydrogels modified with the cell-adhesion peptide RGD activated the appropriate signalling pathways, and over a period of 14 days migrated 85 μm into agarose gels with an immobilized gradient of SHH.

As biomimetic patterning strategies become increasingly complex, it is likely that one may need to simultaneously employ many orthogonal chemical-modification strategies to generate biochemical complexities that rival the ECM. The findings by Wylie and colleagues[1] may guide development of increasingly elegant ECM-mimetic materials, incorporating numerous bioactive agents in complex spatial arrangements and ultimately advancing our capabilities in tissue engineering and regenerative medicine. Furthermore, as new tools continue to be developed for biomimetic patterning, one can even foresee a time when we will be able to take images of tissues or cells and directly translate these to biomimetic patterns in synthetic hydrogels to more truly recapitulate the physiological microenvironment. ❐

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References

1. Wylie, R. G. *et al. Nature Mater.* **10,** 799–806 (2011).
2. Abbott, A. *Nature* **424,** 870–872 (2003).
3. Hern, D. L. & Hubbell, J. A. *J. Biomed. Mater. Res.* **39,** 266–276 (1998).
4. Mann, B. K., Schmedlen, R. H. & West, J. L. *Biomaterials* **22,** 439–444 (2001).
5. Moon, J. J., Lee, S. H. & West, J. L. *Biomacromolecules* **8,** 42–49 (2007).
6. West, J. L. & Hubbell, J. A. *Macromolecules* **32,** 241–244 (1999).
7. Denk, W., Strickler, J. H. & Webb, W. W. *Science* **248,** 73–76 (1990).
8. Hoffmann, J. C. & West, J. L. *Soft Matter* **6,** 5056–5063 (2010).
9. Kloxin, A. M., Kasko, A. M., Salinas, C. N. & Anseth, K. S. *Science* **324,** 59–63 (2009).
10. Scadden, D. T. *Nature* **441,** 1075–1079 (2006).
11. Zaidel-Bar, R., Cohen, M., Addai, L. & Geiger, B. *Biochem. Soc. Trans.* **32,** 416–420 (2004).

PHOTOEMISSION SPECTROSCOPY

Deep into the bulk

The electronic structure in the bulk of a crystal can be unveiled by hard X-ray angle-resolved photoemission spectroscopy.

Dong-Lai Feng

Over the past 50 years, angle-resolved photoemission spectroscopy (ARPES) has developed into a powerful technique for studying the electronic structure of crystals. High-temperature superconductors[1], graphene[2] and topological insulators[3] are just a few examples of materials that have been investigated with this technique, which makes full use of the photoelectric effect, in which an electron is emitted from a solid when it is excited by a photon. Albert Einstein correctly postulated the relationship between the kinetic energy of the photoelectron, E_{kin}, and the frequency, ν, of the photon, $E_{kin} = h\nu - E_b$, where h is Planck's constant and E_b is the electron binding energy, and helped lay down the foundation of quantum mechanics. In parallel, for emission from solids with crystalline order, there is a conservation relationship between the photoelectron momentum and the momentum of the electron in its original state in the solid. Therefore, by measuring the momentum, energy, and perhaps also the spin of photoelectrons, one can deduce the electronic states in the solid and understand its properties.

Although ARPES has played an important role in condensed-matter physics research, its capability is usually limited by the low inelastic mean-free path (IMFP) for the photoelectron to escape without an additional loss of energy and change of momentum[4]. In the typical ARPES experiment with photon energies of ~10–100 eV, the probing depth is only about 5–10 Å, and surface effects are often dominant. One way around this problem