Singh et al. is that these additional electrons do not join in the existing superconducting condensate but form a second one[1]. To our knowledge, this tunable switching between a single- and a multi-condensate superconductor is a premier result in the field of superconductivity. Remarkably, it does not seem to occur at the (001) interface, studied previously by some of the same authors, which showed single condensate behaviour under varying band occupancies[8].

A second surprise reported by Singh et al. is that the emergence of the second condensate weakens the overall strength of the superconductivity. Measurements of the superfluid stiffness — that is, the rigidity of the superconducting state against fluctuations in the phase of its wavefunction — indicate that these fluctuations can proliferate easier. This softens the superconductivity and reduces the critical temperature[1]. These conclusions are based on microwave-resonance experiments, in which the kinetic inductance of the superconductor is probed. Like for the electrostatic tunability of superconductivity, the low superfluid density of $SrTiO_3$ is the key enabler for such experiments, as the kinetic inductance of a superconductor is inversely proportional to that density. We note here that such microwave-resonance experiments can also be of great interest in the characterization of other low-density, low-dimensional superconductors, including the recently discovered magic-angle twisted bilayer graphene[9]. Moreover, the high kinetic inductance and the sensitivity of these materials to changes in the Cooper pair density hold promise for applications in single-photon detectors: a single photon breaking up a Cooper pair should result in a well-detectable shift of the resonance frequency of the microwave circuit.

Based on the gate dependence of the superconducting properties, Singh et al. conjecture that the multi-condensate superconductivity at these (110)-oriented interfaces has an unconventional $s^{\pm}$ symmetry of the order parameter (Fig. 1d), attributed to a repulsive coupling between the bands involved[1]. With this, the two condensates would be characterized by an opposite phase in their combined superconducting wavefunction. When thinking about 'smoking gun experiments' to further substantiate the present findings and test for an $s^{\pm}$ symmetry, tunnelling spectroscopy studies quickly come to mind. Such experiments can provide further evidence for the formation of a double condensate by displaying two different gaps[3] and can reveal additional details on the superconducting state. A special type of tunnel contact is a Josephson junction, in which both electrodes are superconducting. Such devices could allow phase-sensitive measurements, confirming the sign changes in the superconducting order parameter associated with the $s^{\pm}$ order parameter symmetry, similar to earlier work on the iron pnictide superconductors[10].

Altogether, the work by Singh et al. provides interesting and useful pieces to the puzzle of understanding the properties and mechanisms of unconventional superconductivity in oxide-based systems, of which the most paramount challenge remains unravelling the long-standing mystery of high-T_c superconductivity in the cuprate perovskites. ❐

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PROTEIN PATTERNING

Moving hydrogels to the fourth dimension

Materials that permit spatiotemporal control of biomolecule presentation have long been a challenge in the field. A method has now been developed to reversibly pattern cell-laden hydrogels with site-specifically immobilized proteins using sortase-mediated transpeptidation without compromising bioactivity.

Jonathan H. Galarraga and Jason A. Burdick

With advances in new material chemistry and processing, biomaterials have evolved in the past few decades from static and inert to dynamic and instructive[1]. This evolution has included the development of three-dimensional materials that change over time — the fourth dimension — such as with stimuli-responsive materials that sense extrinsic or intrinsic signals (for example, light and enzymes), shape-memory polymers that revert from a temporary to permanent shape, or degradable materials whose properties change with material erosion. Hydrogels, or water-swollen polymer networks, are of particular interest in biomedical fields since they permit encapsulation of cells and can be engineered with a plethora of signals that mimic features of the native extracellular matrix (ECM). Numerous approaches have been introduced in recent years to spatiotemporally control hydrogels; yet, the development of techniques to manipulate important full-length proteins with such control has been challenging. DeForest and colleagues now report in *Nature Materials* on a method where full-length proteins can be incorporated into hydrogels without compromising their bioactivity, such that their presentation to encapsulated cells can be modulated with spatiotemporal precision and subcellular resolution[2].

Biological processes such as morphogenesis, tissue development and disease progression are complex, and cells receive numerous spatiotemporal biochemical and biophysical signals from their surroundings to control their behaviour. It is now well understood that cells are influenced by signals in their microenvironment such as proteins,

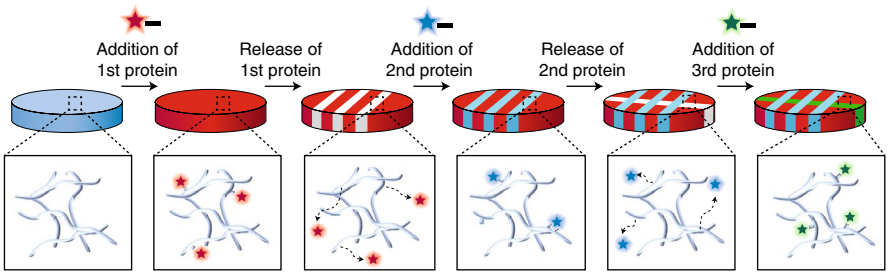

Fig. 1 | Photopatterning and photorelease of sortase-tag enhanced protein ligation (STEPL)-modified proteins in hydrogels. Full-length proteins of interest are first modified with linker functional groups via sortase A-mediated transpeptidation. Thereafter, proteins are immobilized within polymer networks on irradiation with light and can later be photoreleased using alternative chemistry. These steps can be repeated for the controlled addition and release of multiple proteins within a single hydrogel, providing spatiotemporal control over protein presentation to cells.

small molecules, ECM mechanics and communication with neighbouring cells[3]. It is desirable to use hydrogels that encompass such signals for either in vitro platforms to better understand biological signalling in these processes, or to control cell behaviour towards the design of translational therapies (for example, tissue engineering). A variety of techniques have emerged to control these signals and introduce desired heterogeneity into materials, such as photopatterning to introduce ligands with spatial control[4] or photodegradation reactions to soften hydrogels over time[5]. Much less work has been conducted on controlling full-length proteins, probably due to their added complexity in maintaining function throughout processing; however, there are examples where photopatterning was used sequentially to introduce multiple proteins into a hydrogel[6] or where bio-orthogonal photochemistry was used to introduce and then release proteins from hydrogels[7].

Incorporation of proteins into hydrogels has traditionally relied on random installation of simple linker groups, a non-specific process that often attenuates protein bioactivity and stability. DeForest and colleagues report on the use of sortase-tag enhanced protein ligation (STEPL) to modify proteins of interest with linker groups in a precise and site-specific manner, rendering them amenable to photopatterning into poly(ethylene glycol) (PEG)-based hydrogels[2]. The enzyme sortase A, which is found in the bacterium *Staphylococcus aureus*, exhibits the ability to mediate transpeptidation, a process through which one or more amino acid residues are transferred between two distinct peptides[8]. DeForest and colleagues leveraged STEPL to attach customizable peptide probes containing a variety of material-reactive handles onto proteins of interest with recombinant technology, developing a library of modified proteins that included fluorescent proteins, enzymes and growth factors. Critically, these proteins retained native bioactivity while also possessing the functional handles requisite for material decoration.

With these modified proteins in hand, DeForest and colleagues incorporated them into PEG hydrogels using cycloaddition reactions for gelation and then photomediated oxime ligation for protein immobilization with spatial control[2]. The incorporated proteins could be subsequently released with light through ortho-nitrobenzyl ester chemistry. This process of protein ligation and release could be repeated sequentially in arbitrary four-dimensional space (Fig. 1). It was shown that β-lactamase, a model enzyme, could be photopatterned to preferentially localize enzymatic activity within a hydrogel. Growth factors, including epidermal growth factor and fibroblast growth factor, were also patterned within hydrogels and subsequently released on irradiation with light, permitting temporal control over cellular uptake and downstream response. These examples illustrate unprecedented spatiotemporal control over active protein presentation within hydrogels.

Despite the overall elegance of the presented approach, one potential challenge lies in its complexity, as specialized techniques (for example, multiphoton laser-scanning lithography) and expertise in small-molecule and peptide synthesis, protein engineering and applied photochemistry are rarely present within an individual laboratory. Such complexity limits accessibility to the biologists who would benefit most from these advanced techniques, and potentially dampens enthusiasm from industry striving for translational materials where simplicity is often desired over complexity[9]. Furthermore, while the ability to pattern and release STEPL-modified proteins with orthogonal light-based reactions is promising for mimicking the dynamic presentation of proteins observed in ECM, the relatively slow diffusional processes necessitated by this approach may not capture the timescales of many relevant, endogenous signalling events and cells may already be changing their own environments over time through the deposition of proteins[10]. These limitations are likely to be overcome with advances in the chemistry used, reagent commercialization and further exploration of the technology.

It will be very exciting to see the innovations that are made with this new technique, as 'top-down' control over the presentation of proteins within hydrogels is now possible to address complex biological questions and to modify cell behaviours, particularly in three-dimensional environments. The biological questions and applications that can harness such technology to match the complexity of cell signalling (through temporal molecule control) and the heterogeneity of cellular structures (through spatial control) are endless. ❐

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