techniques, or producible only by using processes that exhaust both time and resources. The authors' method can also make particles with surface features as small as 4 $\mu m^2$, offering the potential to create extremely intricate microfeatures of high quality.

The r2rCLIP technique is compatible with a wide range of printable materials, including standard polymers, ceramics and hydrogels. Ceramic materials are attractive for many applications, such as functional microcomponents in the smallest precision instruments and in microelectromechanical systems that are used in the electronics and telecommunications industries. The applicability of the authors' method to the mass production of hydrogel particles could have an impact on drug delivery and on other bioengineering technologies. One key factor for these biomedical applications is the versatility and uniformity of the particles produced, as well as the structural precision with which they can be manufactured. These features make the authors' approach well suited to therapies that require specific particles to distribute drugs through different means; for example, by injection or inhalation.

Kronenfeld and colleagues' technique is remarkable in its performance and in the quality of the particles it produces. The development of custom-designed materials for r2rCLIP might be the next key step. In particular, the authors' method could have a pronounced effect on a wide range of fields, including biomedicine and robotics, if it were integrated with materials that exhibit smart features, such as the ability to interact with the surrounding environment.

In devising r2rCLIP, Kronenfeld *et al.* have provided academia and industry with an excellent strategy for manufacturing microparticles, which is impressive in terms of the versatility of the shapes it produces and the materials it can use. The technique is also superior to existing approaches in terms of the resolution of intricate features it can engineer, the creation of complex geometries and the speed and volume of the manufacturing process. It will be exciting to see the ways in which this technology can improve production processes in many areas of science and technology.

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Immunology

# Interactions between immune cells recorded

**Michael A. Wheeler**

Direct interactions between cells in tissue are incompletely understood because the advanced technologies required to examine them are still in their infancy. A new method can decipher cell–cell interactions on a large scale. **See p.399**

In living tissues, millions of cells communicate with each other within fractions of a second. How are these dynamic intercellular interactions orchestrated? On page 399, Nakandakari-Higa *et al.*[1] present a method called universal LIPSTIC (uLIPSTIC) that offers a way to address the challenge of investigating transient interactions between cells of the immune system that come into close physical proximity. This technique can also be adapted to study interactions between other cell types.

During sickness, a remarkable molecular dialogue occurs across the body through the release of immune-signalling proteins called cytokines. A spatially distributed set of immune cells acts to translate these signals and drive host defence responses. Effective immune-system action requires the precise coordination of a vast array of interacting cell types. In some cases, these interactions include physical contacts between interacting immune cells. Such contacts generate molecular complexes called immunological synapses at the interface of these cells, which are required for responses to harmful agents (pathogens).

**"The authors have demonstrated the broad utility of this technique across tissues, cell types and immune challenges."**

Although experiments first identified where an immunological synapse is located on the surface of two interacting cells more than 26 years ago[2], several questions remain to be answered. In particular, how does the diversity of immune responses in the body arise through exquisitely specific, yet transient, interactions between cells?

Specialized contacts similar to those of the immunological synapse are made by other interacting cell types throughout tissues. A growing number of tools are being developed to study cell communication in various organs[3], and these methods involve a combination of techniques, such as high-throughput genomics[4–6], proximity-based labelling by enzymes[7], microfluidics[8] and bioinformatics[9]. But none of these methods is appropriate for addressing the paradoxical communication scenario of immune cells, which are predisposed both to contacts on the cell surface and to frequent cellular turnover.

To find a way forward, Nakandakari-Higa *et al.* capitalized on a technology called LIPSTIC ('labelling immune partnerships by sortagging intercellular contacts') that was previously developed[10] by members of the same team. The method exploits a low-affinity interaction between two molecules: a bacterial enzyme called SrtA on the surface of a donor cell; and its target, a stretch of five glycine amino-acid residues ($G_5$) on the surface of an acceptor cell. SrtA catalyses the transfer of a peptide substrate — attached to a molecular label called biotin — to $G_5$, which is part of a protein on the acceptor-cell surface (Fig. 1).

Cells labelled with biotin are inferred to have interacted with a donor cell because the peptide substrate can be transferred from SrtA-expressing to $G_5$-expressing cells only when the local concentration of peptide substrate is high enough to result in the occurrence of the otherwise low-affinity interaction between SrtA and $G_5$. Physical contacts at the immunological synapse fulfil this criterion because the estimated distance between cellular membranes is approximately 15 nanometres — a distance so small that any quantity

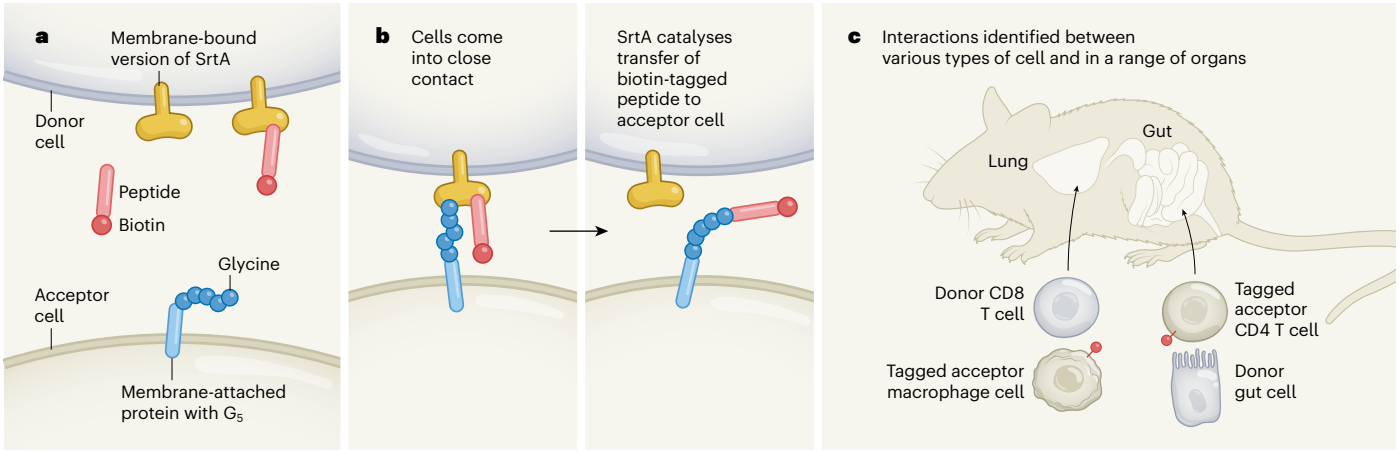


**Figure 1 | A way to track direct physical interaction between cells.** **a**, Nakandakari-Higa *et al.*[1] present a method called uLIPSTIC that identifies cells that come into close proximity. The authors engineered mice so that selected lineages of 'donor' cells express an engineered membrane-bound enzyme called SrtA. SrtA can catalyse an interaction that joins a peptide tagged with the molecule biotin to a stretch of five glycine amino-acid residues (termed $G_5$). The $G_5$ is attached to a membrane protein on cell lineages engineered to be 'acceptor' cells. **b**, If the donor and acceptor cells come into close contact, this interaction generates tagged acceptor cells that can be identified by means of the biotin tag. **c**, The method has wide potential for identifying interactions between different types of cell across a variety of tissues. The authors identified these interactions between various types of immune cell and a gut cell in mice.

of peptide substrate in the space between cells becomes relatively abundant, thus increasing the likelihood of transfer. However, the original LIPSTIC method[10] was restricted to cells that expressed specific proteins (CD40 and CD40L) found at immunological synapses. Using a more general approach to track the contacts between cells might offer a way to address a broader variety of questions in immunology.

To develop such a method, Nakandakari-Higa *et al.* engineered mice in which both the SrtA and $G_5$ components of the LIPSTIC system were expressed in a mutually exclusive manner whereby cells that expressed $G_5$ did not express SrtA, and vice versa. All cells in an engineered mouse that ubiquitously expressed the engineered $G_5$-tagged protein, and did not express SrtA, did not 'donate' peptide substrate, indicating that in the absence of SrtA, the biotin tag is not transferred to $G_5$. By using sophisticated genetic-engineering approaches in mice, Nakandakari-Higa and colleagues could then bestow donor status to, and revoke acceptor status from, selected cell lineages, enabling them to map cellular contacts that were made only by narrowly defined classes of cell. In contrast to the original LIPSTIC system, in which SrtA and $G_5$ were joined to the proteins CD40 and CD40L, uLIPSTIC generates membrane-bound forms of SrtA and $G_5$ by joining these components to small sections of proteins localized to the cell surface. In principle, uLIPSTIC can be applied to any cell contacts where surface–protein interactions take place.

The authors demonstrated that the interactions detected by uLIPSTIC were physiologically relevant, first by returning to well-defined interactions at the immunological synapse as a proof-of-concept of the system. By modifying T cells of the immune system to target only one peptide antigen (a protein fragment that drives an immune response), the authors showed that interactions detected by uLIPSTIC at immunological synapses were limited to cells that were capable of recognizing this antigen. Crucially, perturbing key molecules that underlie the organization of immunological synapses meant that uLIPSTIC was no longer able to detect the antigen-specific interactions at these synapses.

Nakandakari-Higa and colleagues also showed that uLIPSTIC can be used to study questions about the spatial and temporal dynamics of cell communication in the immune system across different cellular activation states. For instance, the authors used uLIPSTIC to determine the interactions of a comparatively rare subset of immune cells, called regulatory T cells, that has a role in immunosuppression. This suggests that uLIPSTIC can detect interactions even in specialized populations of cells. The authors also profiled cell–cell interactions that are restricted in space and time and that initiate the production of antibodies. These results identified known cellular partners that initiate the process of antibody generation, thus demonstrating that uLIPSTIC captures interactions associated with transient, local cell–cell communication.

To push the limits of uLIPSTIC and comprehensively study communication between cells of the immune system, Nakandakari-Higa *et al.* combined their method with single-cell RNA sequencing, a high-throughput genomic technique that provides cell-state information at the level of a single cell. The authors evaluated the scale of peptide transfer from donor to acceptor cells by measuring the levels of uLIPSTIC-transferred biotin on the cell surface, and then correlated the uLIPSTIC signal with gene expression as assessed by single-cell RNA sequencing. This approach provides insight into the molecular mechanisms that govern the strongest cell–cell interactions, which would not be identified in conventional analyses. By analysing immune-cell interactions in the gut and during viral infection, uLIPSTIC revealed interactions, at the single-cell level, not only in different classes of immune cell but even with neighbouring non-immune cells in the gut, indicating that this method can be used in a variety of tissues.

How might uLIPSTIC shape the frontiers of the cell–cell communication field? On the one hand, the authors have demonstrated the broad utility of this technique across tissues, cell types and immune challenges, which speaks to the ability of uLIPSTIC to capture a range of cell interactions that are relevant for biology. On the other hand, the extent to which cell contacts must mimic features of the immunological synapse — such as the distance between interacting cells or the molecular machinery involved — to be captured by uLIPSTIC remains unclear.

Nevertheless, Nakandakari-Higa and colleagues' data suggest that this tool has general potential for use in a variety of cells. As evidence of this, the authors detected substantial uLIPSTIC signals of biotin poised for transfer by SrtA-expressing donors in a class of brain immune cells in healthy mice. In such animals that lack disease-causing agents, not only are the features of the immunological synapse absent, but the molecular machinery to form immunological synapses is expressed only at low levels[11]. Moreover, the range of distances between physically connected cells in the brain (around 3–40 nm) is distinct from that observed in the immunological synapse (approximately 15 nm).

These findings suggest that uLIPSTIC might

be widely adopted by other fields of study. Thus, exciting possibilities exist for uLIPSTIC to become a standard tool to advance the study of cell–cell communication in the immune system and beyond.

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Neuroscience

# Non-neuronal brain cells modulate behaviour

**Anna Kruyer**

A single gene in astrocytes can constrain repetitive behaviours, indicating that these cells are regulators of behavioural disruption in conditions such as Huntington's disease and obsessive–compulsive disorder. **See p.358**

Neurons have long been considered the brain cells chiefly responsible for behaviour, but this longstanding perspective overlooks an important cell type — the astrocyte. On page 358, Ollivier *et al.*[1] report that a subpopulation of brain astrocytes can control repetitive behaviors. The authors' findings make a compelling argument for considering astrocytes as a crucial regulator of behavioural disruption in various cognitive and psychiatric disorders.

Astrocytes are a diverse type of glial cell, whose name refers to its inferred function as 'glue' that holds neurons and other brain cells in place. Consistent with this moniker, astrocytes and other glia are mostly considered supporting cells for neurons, which are given exclusive credit for encoding the brain's fundamental functions: thinking and controlling behaviour. Emerging research contends with this assumption by showing that astrocytes play an important part in modulating signalling between neurons at junctions called synapses. Thus, astrocytes are elevated from neuronal glue to puppeteers of neuronal function, and key drivers of behaviour.

Ollivier *et al.* describe a subpopulation of astrocytes, notable for their expression of the gene *Crym*, in a portion of the brain called the striatum that encodes motivation and habit. Although the protein that *Crym* encodes, μ-crystallin, was discovered in 1957 (ref. 2), its role in the brain has remained mostly unexplored until now.

Interest in the function of μ-crystallin in the brain was spurred by genetic studies that demonstrated a relationship between the *Crym* gene and seemingly disparate brain disorders. In obsessive–compulsive disorder (OCD) and Huntington's disease (HD), for example, *Crym* expression is inversely correlated with disease severity[3,4]. Ollivier and colleagues' careful examination of μ-crystallin localization revealed that its dense expression in the striatum is targeted not to neurons, but to a population of astrocytes. They used animal models to explore the behavioural consequences of *Crym* downregulation in striatal astrocytes, as observed in people with OCD and HD.

Using a series of behavioural tests, the authors report that an artificially reduced expression (knockdown) of *Crym* has no effect on motor control or anxiety, but produces a striking increase in 'perseveration', or repetitive behavioural patterns that serve no apparent purpose. In rodents, perseveration reveals itself through an increase in

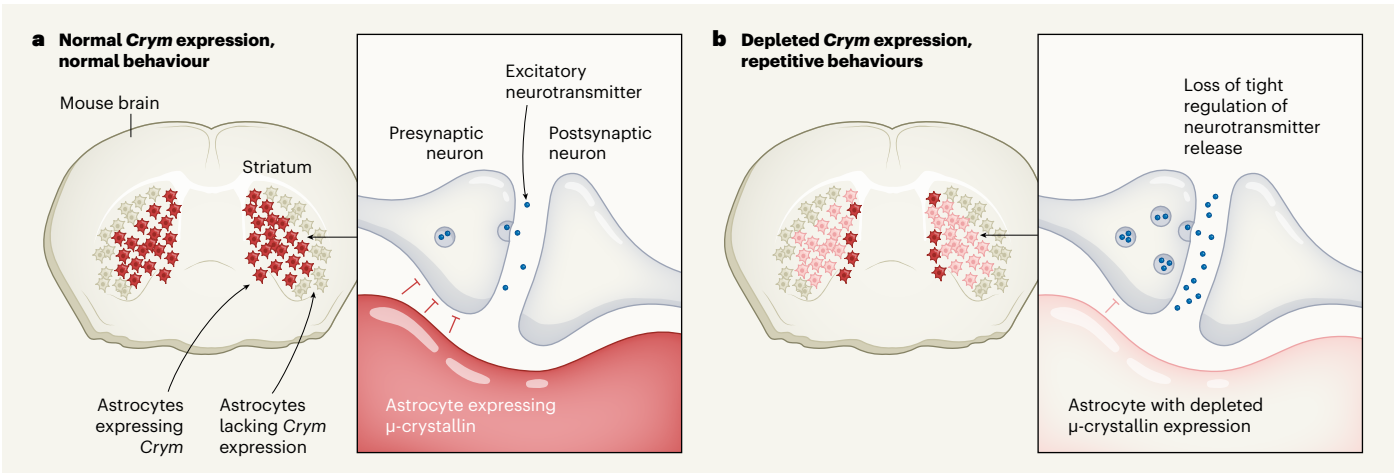


**Figure 1 | Signalling between brain astrocytes and neurons permits flexible, rather than repetitive, behaviours.** **a**, In a part of the mouse brain that controls motivation and habit (the striatum), Ollivier *et al.*[1] discovered a subpopulation of non-neuronal cells, known as astrocytes, that is distinctive for its dense expression of the gene *Crym*, which codes for the protein μ-crystallin. They found that *Crym*-expressing astrocytes (red) contribute to the tight control of the release of excitatory neurotransmitter molecules at synapses (junctions between neurons), and hence to normal behaviour in mice. **b**, When the authors artificially depleted *Crym* expression in striatal astrocytes (pink), they observed a disinhibition of excitatory neurons and an increased release of excitatory neurotransmitter molecules, leading to neuronal hyperactivity. *Crym* depletion resulted in a lack of behavioural flexibility in mice, leading them to engage in repetitive behavioural patterns such as excessive self-grooming.