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SPOTLIGHT

Easy and robust micropatterning using fibrinogen anchors

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Micropatterning is a process to precisely deposit molecules, typically proteins, onto a substrate of choice with micrometer resolution. Watson et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202009063) describe an innovative yet accessible strategy to enable the reproducible micropatterning of virtually any protein while maintaining its biological activity.

Micropatterning involves depositing molecules, primarily proteins, in a precise pattern on a defined substrate. Since the first applications of micropatterning in biology in the 1970s (1), this technique has become increasingly adopted and led to a variety of important biological discoveries related to the impact and mechanisms of cellular architecture and subcellular cytoskeletal components on cell function (2, 3, 4). The basis of most micropatterning strategies has remained the same for decades: a substrate is coated with a specific, often micron scale, pattern of protein within a background of an anti-fouling agent. This can be achieved in two ways: the substrate is coated with the anti-fouling agent, which is then locally removed in a precisely defined pattern using light projection through a mask, and protein is then deposited in regions free of the anti-fouling agent. Alternatively, protein can be directly printed onto the substrate, for example using an elastomeric stamp, or by photochemical linking via patterned light, and then unpatterned substrate regions are backfilled with the anti-fouling agent. Protein patterns can then be used to control the architecture of whole cells or the organization of subcellular components.

While micropatterning is potentially a very useful methodology for a number of applications, widespread adoption of this method was initially limited by the need for access to specialized photolithography equipment to generate either the stamps or to directly pattern the anti-fouling layers. Further, since the masks used to define the stamp or anti-fouling layer patterns are pattern specific, a new mask must be produced for every pattern alteration. Early micropatterning methods also were not ideal for creating patterns containing multiple proteins, as sequential protein stamping or sequential anti-fouling coating removal required tedious and time-consuming stamp or mask alignment to ensure accurate patterning. To overcome these two limitations, Strale and colleagues developed a micropatterning technique called LIMAP (light-induced molecular absorption), in which a UV laser within a microscope is used to photoexcise patterns in the anti-fouling layer without the need for a mask. Further the use of the microscope enables direct visualization of protein patterns and hence easy alignment during multiprotein patterning (5). Despite the increased accessibility achieved by this advance, a number of other challenges still limit the widespread application of LIMAP for micropatterning (Fig. 1). First, pattern robustness remains a challenge, as a proportion of protein attaches outside of the pattern in the anti-fouling region (patterns lacks specificity) and protein distribution on the pattern is not even (pattern lacks homogeneity). Second, maintaining the biological activity of the proteins printed can be a challenge, as LIMAP does not enable control over protein-substrate interactions and the orientation of the deposited protein on the substrate, which can result in inaccessibility of the protein's biologically active domains. This second limitation in particular dramatically limits the types of protein that can be printed and hence cell types and biological questions that can be explored using the LIMAP technique.

In this issue, Watson and colleagues (6) present a new approach that overcomes these limitations by using fibrinogen anchors, functionalized to bind to any proteins of interest, to enable robust micropatterning of almost any combination of proteins. Specifically, as a first step, LIMAP was used to photopattern fibrinogen molecules conjugated to a specific binding target. In a second step, the protein of interest displaying the corresponding binding partner was then added, under appropriate buffer conditions to maintain protein activity, and bound preferentially to the fibrinogen anchors (Fig. 1). The authors describe generation of a number of conjugates, based on modification of fibrinogen-exposed amines, that enable application of the method to a number of proteins, including fibrinogen–Con A, a lectin that binds to insect cells, fibrinogen-NeutrAvidin to bind to biotinylated targets, fibrinogen-GBP to bind to GFP-tagged proteins (GBP stands for GFP-binding peptide, a nanobody against GFP), and fibrinogen-biotin to bind

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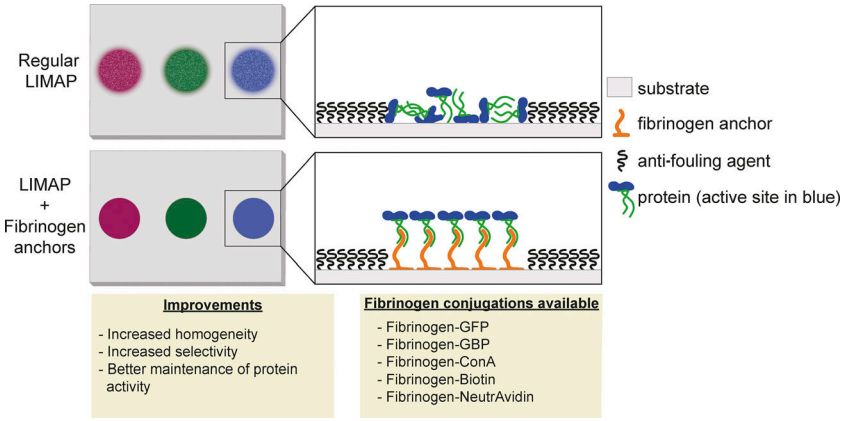

Figure 1. **Overview of fibrinogen anchor micropatterning strategy and advantages over existing patterning techniques.**

to streptavidin/NeutrAvidin fusions, as well as to biotinylated targets using a NeutrAvidin sandwich (fibrinogen-biotin::NeutrAvidin::biotinylated protein of interest). Of note, the simplicity of this conjugation process will enable greater adoption of this approach by the biology community.

Fibrinogen was selected as the anchor molecule as its properties are such that homogeneous and specific patterning can be routinely achieved using LIMAP. By binding other proteins to the fibrinogen anchor, the authors demonstrate their method can produce micropatterns of the anchor bound with the same high specificity (proteins only pattern where they should) and high homogeneity (protein distribution within the pattern is less variable). Further, the authors take advantage of the library of different fibrinogen conjugates to generate patterns containing three different proteins that bind to three different sequentially patterned fibrinogen conjugates. While the improvements in selectivity and homogeneity achieved by Watson et al. are alone a significant step forward, the most exciting demonstrations in their study is that their method enables maintenance of the biological activity of printed proteins in a number of different contexts (6). For example, the authors pattern proteins to perform a microtubule gliding assay and show that printed kinesin motors maintain their ability to move microtubules with the expected gliding speed and dynamics. In another example, the authors exploit their ability to pattern multiple proteins at the subcellular scale and demonstrate that patterns of EGF can induce relocalization of the corresponding EGFR receptor only to regions of a cell attached to EGF-patterned regions on the substrate.

The simplicity of the described approach and compatibility with different proteins make it attractive to explore a broad range of problems in the future. At the multicellular scale, this approach could be applied to understand localized cell–cell interactions during collective phenomena such as organoid patterning and models of early human development (7), cell competition dynamics (8), or collective cell migration (9). In particular, applying sequential patterning to create heterogeneous signaling within a cellular island would be interesting to explore developmental patterning circuits. At the single-cell scale, this approach could be useful for understanding heterogeneity in cell populations, although the patterning throughput achievable using microscope "writing" could limit the number cells that can be assessed for such applications. Integration of this anchoring approach with higher throughput mask-based micropatterning methods could address this, however. Perhaps most excitingly, this approach offers a powerful strategy to broadly control subcellular features in a cell. For example, this opens the possibility to probe which downstream networks are activated upon receptor engagement and to control the spatial locations of signaling components within a cell (10). This clever addition to the micropatterning tool kit significantly expands the types of problems that micropatterning can be used to explore while maintaining the accessibility of the technique to the biological community.

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