to efficient single carbon-based bioprocesses.

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Spotlight

Stepping into the Next Dimension of Biomaterial Design

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Recapitulating the dynamic spatiotemporal behavior of the extracellular matrix using biomaterials is an ongoing challenge. A recent breakthrough by Shadish *et al.* demonstrates the use of sortase-mediated transpeptidation to site-specifically modify proteins with a range of chemical motifs. Modified proteins were immobilized within biomaterials with high spatiotemporal control and resulted in localized bioactivity.

The biophysical and biochemical signals within the extracellular matrix (ECM) are finely regulated and constantly changing with time. The dynamic nature of these signals is critical for development, wound healing, regeneration, maintaining homeostasis, and disease progression [1]. Biomaterials that are capable of recapitulating the ECM are needed to create *in vitro* tissue and disease models, as well as design tissue-engineered scaffolds that can finely control multistep biological processes for functional tissue regeneration.

Recent technological advances have enabled the development of complex biomaterials that can begin to mimic the dynamic nature of the ECM. These advances include the development of new manufacturing techniques for increased spatial control over material properties, such as microfabrication, photopatterning, lithography, and three-dimensional bioprinting [2]. Additionally, the incorporation of stimuli-responsive chemical motifs allows for changes in material properties with time, moving biomaterial design into the fourth dimension [3,4].

Despite these advances, the incorporation of full-length proteins within biomaterials remains a challenge. Protein signaling is known to influence a number of complex biological processes, including cell fate and new tissue formation. Protein immobilization methods have included chemical, enzymatic, and noncovalent conjugation techniques, where the conjugation strategy is critical in determining protein bioactivity and efficacy can vary between specific proteins [5]. Furthermore, proteins have traditionally been immobilized within biomaterials using nonspecific conjugation reactions, which can lead to unpredictable and significant decreases in protein bioactivity.

Shadish and colleagues recently reported an innovative approach to reversibly pattern full-length proteins with subcellular precision while maintaining high levels of protein bioactivity [6]. Full-length proteins were modified in a precise and site-specific manner using an enzymatic conjugation technique, sortase-tag enhanced protein ligation (STEPL) [7]. The enzyme sortase A, present in *Staphylococcus aureus*, enables protein modification via transpeptidation. Briefly, sortase A recognizes a specific amino acid sequence (or sorting signal), which can be displaced for a peptide sequence that contains a reactive chemical functionality [8]. Shadish and colleagues used STEPL to create a library of homogeneous, site-specifically modified proteins with different photoreactive handles. The modified proteins included fluorescent proteins, enzymes, and growth factors, where all proteins maintained high levels of bioactivity following modification.

To demonstrate the modified proteins can be controllably incorporated into biomaterials, this study used a poly(ethylene glycol) (PEG)-based hydrogel system containing photocaged reactive alkoxyamine functional groups. Proteins modified with an aldehyde functional group will react with uncaged alkoxyamine via oxime ligation resulting in covalent protein immobilization within the hydrogel. For removal, proteins were modified with an ortho-nitrobenzyl (oNB) ester functional group that photodegrades in the presence of ultraviolet light. Since both protein addition (i.e., uncaging of the alkoxyamine) and protein removal rely on ultraviolet light, protein immobilization can be spatially controlled using a number of techniques, including: mask-based photolithography to generate two-dimensional patterns, a sliding opaque

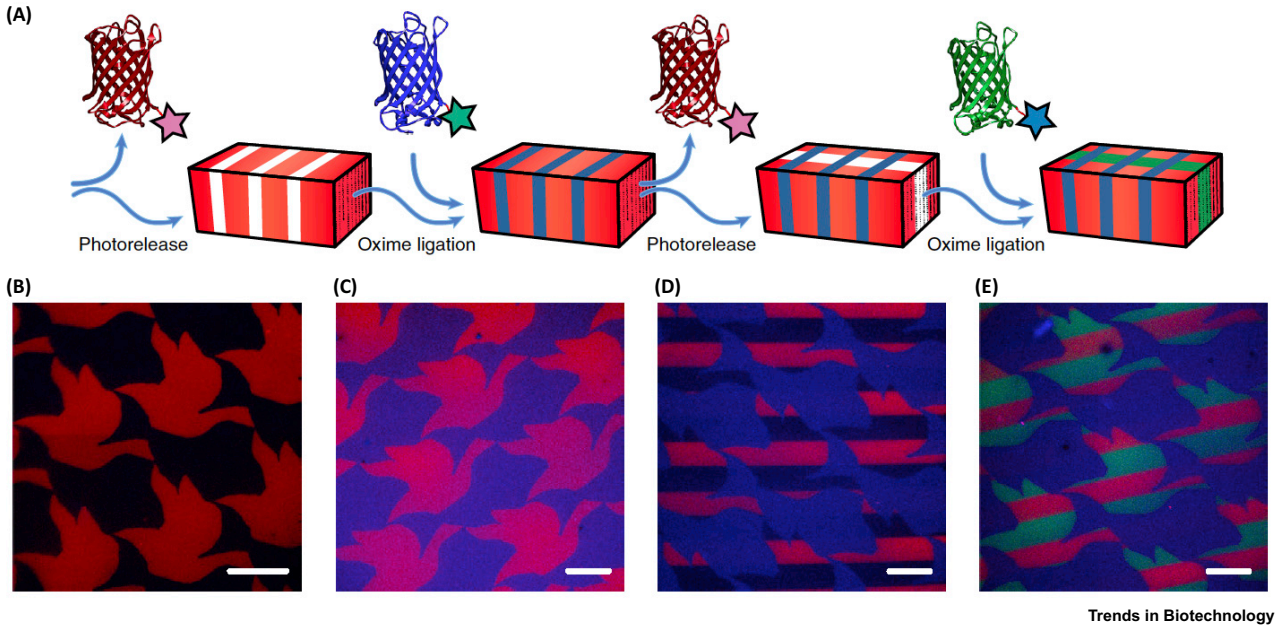

Figure 1. Sortagged Proteins Enable Four-Dimensional Control over Protein Presentation.
(A–E) Sortase-tag enhanced protein ligation (STEPL)-modified proteins can be added (via oxime ligation or other chemistries) and removed (via photorelease) to spatially and temporally control protein presentation. These steps can be repeated over multiple cycles and for multiple proteins within a single material. Reproduced, with permission, from [6].

photo mask to generate two-dimensional gradients, and multiphoton laser-scanning lithography to generate complex three-dimensional shapes. Using this approach, multiple proteins can be added and removed with a high degree of spatial control and at user-defined times (Figure 1).

Next, Shadish and colleagues demonstrated immobilized proteins retain their bioactivity and resulted in localized activity. The enzyme β-lactamase, known to hydrolyze thioacetate cefalotin and result in a precipitate, was modified via STEPL and immobilized within PEG hydrogels. In the presence of thioacetate cefalotin, precipitate formation was only observed in β-lactamase-immobilized hydrogel regions. Similarly, STEPL-modified epidermal growth factor (EGF) containing the photosensitive oNB moiety for photoremoval was immobilized within PEG hydrogels. Encapsulated cells were able to bind to EGF and resulted in increased localized proliferation. At day three, photoremoval of EGF mitigated initial local effects, likely due to cell migration and redistribution. Interestingly, Shadish and colleagues were able to show that photoreleased EGF was endocytosed into the cell with subcellular precision using multiphoton laser-scanning lithography. Together, this research presents a versatile and powerful strategy for site-specifically modifying full-length proteins to enable immobilization within biomaterials, with unprecedented spatial and temporal control.

Although enzymatic modification of proteins is uniquely capable of selective and site-specific modification, this modification technique is more expensive compared with traditional chemical modification of proteins. Furthermore, widespread adoption may be inhibited by the chemistry and protein engineering expertise needed to create STEPL-modified proteins. Conversely, one can envision a future where STEPL-modified proteins are available commercially with a number of common reactive handles. Lastly, the ability to spatiotemporally control protein presentation will be dependent on protein diffusion in and out of the hydrogel, as well as cell-based deposition of proteins and remodeling [9].

The modular nature of sortase-mediated transpeptidation allows for easy protein modification with virtually any chemical reactive handle and allows for wide-ranging applications in a number of biomaterial systems. Using photoreactive handles, multiple proteins can be added and removed over multiple cycles with a high degree of spatial control,

highlighting the ability of this approach to control protein immobilization in all four dimensions. This level of control over protein presentation will enable both fundamental biological studies, as well as the design of new tissue-engineered materials capable of directing multistep biological processes (e.g., proliferation, differentiation, and new tissue formation). The recent advances by Shadish and colleagues in protein modification and immobilization is truly exciting and will likely transform future biomaterial design approaches.

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Forum

Detecting Harmful Algal Blooms with Isothermal Molecular Strategies

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The use of isothermal nucleic acid amplification strategies to detect harmful algal blooms (HABs) is in its infancy. We describe recent advances in these systems and highlight the challenges for the achievement of simple, low-cost, compact, and portable devices for field applications.

Why Isothermal Molecular Strategies?

Monitoring of harmful algal blooms (HABs) (Box 1) is vital to guarantee the safety and health of coastal environments. Identification and quantification of HAB species has conventionally relied on the observation of morphological characters using a microscope, which is laborious and time consuming. Additionally, microalgal identification is sometimes difficult due to morphological similarities among species. Molecular (nucleic acid) techniques have the principle advantage of highly specific recognition and discrimination of the target species. However, traditional techniques such as PCR still require samples to be sent to centralised laboratories, resulting in a time lag between sample procurement and analysis, increasing the risk of extended contamination. Accordingly, there is an increasing demand for portable and autonomous systems able to perform accurate and rapid analysis in the field. Isothermal amplification techniques can contribute to achieving this objective. Unlike typical thermal cycling amplification, isothermal techniques are carried out at a constant temperature, which reduces the power needed and makes them more compatible for integration into miniaturized systems. Although widely used in clinical diagnostics, the application of isothermal molecular strategies (including isothermal amplification and detection steps) to detect microalgae is still at its preliminary stage.

Isothermal Molecular Strategies for HABs

Among all isothermal techniques described to date, loop-mediated isothermal amplification (LAMP), hyperbranched rolling circle amplification (HRCA), recombinase polymerase amplification (RPA), and nucleic acid sequence-based amplification (NASBA), coupled with different detection strategies, have been successfully used to detect microalgae, with LAMP being the most widely reported technique. In LAMP, 4–6 primers are used to amplify the target double-stranded DNA (dsDNA) at a constant temperature of 60–65°C for 60 min. Single-stranded DNA (ssDNA) LAMP products are a mixture of different lengths with multiple loops and several repeats of the target sequence. LAMP products are generally visualised by a colour change upon addition of a fluorescent intercalating dye, as reported for the detection of *Prorocentrum minimum*, *Karenia mikimotoi*, and *Alexandrium* species [1]. Another strategy to detect LAMP products relies on lateral flow (LF) dipsticks, recently described for *Karlodinium veneficum* [2], *Prymnesium parvum* [3], and *Amphidinium carterae* [4]. In these dipsticks, the use of a biotin-labelled primer results in biotinylated LAMP products that, after immobilization on the strip, hybridize