Facile C-terminal Attachment of Proteins on Surfaces by Hydrazine-Intein Chemical Reaction

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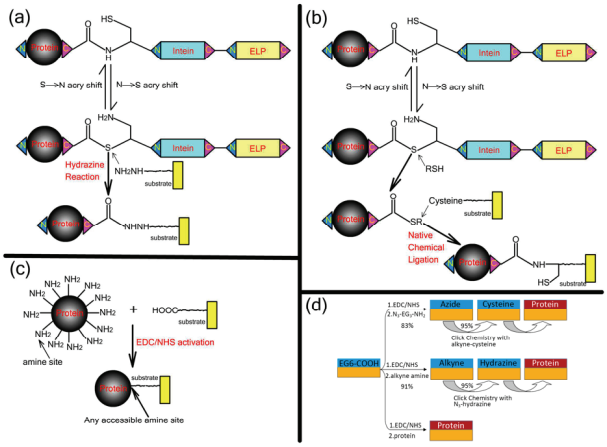
Statement of Purpose: We describe herein, a site-specific protein immobilization strategy that allows control over the orientation of the protein and allows a protein to be directly immobilized from crude cell lyaste. We designed a thioredoxin-intein-elastin-like-polypeptide (Trx-intein-ELP) fusion protein containing an unnatural peptide bond between the C-terminus of the target protein Trx and the N-terminus of the intein. This bond is susceptible to attack by hydrazine, and upon exposing the Trx-intein-ELP fusion protein to a hydrazine-functionalized surface, Trx is immobilized onto the surface via its C-terminus. We also demonstrate that the hydrazine-intein method has distinct advantages over other conventional protein immobilization methods, including higher surface density of immobilized protein, faster reaction kinetics, and the ability to capture target protein directly from complex biological fluids such as cell lysate without purification of the protein.

Methods: A recombinant Trx-intein-ELP (72 kDa) fusion protein was expressed in the E. coli BLR strain and purified by inverse transition cycling (ITC).[1] Protein immobilization was characterized by surface plasmon resonance (SPR, Biacore), ellipsometry (J. A. Woollam), and fluorescence microscopy (Nikon). For the fluorescence microscopy analysis, functionalized surfaces were patterned using microcontact printing (μCP). The target protein was immobilized onto the surface from buffer or from cell lysate. The micropatterns were then incubated sequentially with a Trx-specific antibody and a fluorescent Alexa-IgG secondary antibody to visualize the surface density of the immobilized Trx.

Results: Inteins are reactive towards hydrazine[2] and thiols,[3] and a direct immobilization strategy (Scheme 1a) was designed in which a hydrazine-functionalized surface directly attacks the intein domain of Trx-intein-ELP and couples Trx to the surface at its C-terminus. This method was compared with two other competing methods: native chemical ligation (NCL, Scheme 1b) and EDC/NHS coupling (Scheme 1c). Conventional NCL also attaches a protein to the surface at the C-terminus, but requires cleavage of the Trx from the intein-ELP with exogenous thiols, and the liberated Trx that contains a C-terminal thioester as a consequence of the intein cleavage then reacted with cysteine residues that are presented by the surface to covalently couple the protein (Scheme 1b).

SPR and ellipsometry measurements showed that protein immobilization by the hydrazine-intein and EDC/NHS (Scheme 1c) resulted in similar Trx surface density; however, antibody binding to immobilized Trx was five-fold greater on the hydrazine surface than on Trx coupled by means of NHS/EDC coupling. This difference was attributed to the site-specific immobilization of Trx (protein C-terminus) by the hydrazine-intein method as opposed to the random orientation obtained with the EDC/NHS method. In contrast, SPR and ellipsometry



Scheme 1. Schematic of protein immobilization on a surface by three routes: (a) hydrazine-intein, (b) NCL, and (c) EDC/NHS, and (d) modification steps for each surface.

measurements showed that NCL resulted in a five-fold lower Trx surface density than that achieved with the hydrazine-intein method. The ratio of antibody binding to Trx immobilized by NCL was similar to that observed for the hydrazine-intein method, suggesting that both methods were capable of protein immobilization in a preferred orientation with accessible binding sites to the Trx-antibody, but that the hydrazine attack reaction is more efficient at immobilizing Trx to the surface than conventional NCL. Fluorescence microscopy of micropatterned Trx by the three methods showed that the signal-to-noise for the hydrazine-intein method was almost six times higher than the ratio observed for the other two methods, demonstrating that the hydrazine-intein method provided a better combination of protein immobilization efficiency and antibody recognition as compared to the other two techniques. The hydrazine-intein method could capture Trx on to a surface from cell lysate at concentrations as low as 10 nM, which is almost 1000-fold higher than possible with conventional intein-based NCL.[4]

Conclusions: The surface-mediated hydrazine attack reaction to directly cleave Trx from its intein fusion resulted in a higher surface density, faster binding kinetics, and better sensitivity than other protein immobilization techniques. Furthermore, this method also allows an intein fusion protein to be directly immobilized from crude cell lysate at low protein expression levels.

References:

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