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Facile Chemical Functionalization of Antibodies Through Intein-Linked Yeast Display

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The capability to append unique chemical functionalities to proteins is valuable for a variety of applications including protein immobilization, therapeutic drug delivery, and imaging. One approach that has been employed for site-specific protein functionalization is the reaction of intein fusion proteins with nucleophiles possessing custom chemical properties. While traditional techniques have used soluble, purified fusion proteins, we have extended these methods to release and modify intein fusion proteins expressed on the yeast surface, thereby eliminating the need for soluble protein expression and enabling such modifications to be performed directly from a protein engineering platform. To this end, it is possible to simultaneously release yeast surface displayed proteins and insert chemical functionality compatible with expressed protein ligation or click chemistry. For instance, single-chain antibodies (scFvs) displayed as fusions to the Mxe GyrA intein on the yeast surface were released and functionalized with an alkyne group at the carboxy terminus. The scFvs were directly immobilized on azide-decorated surfaces in a click chemistry reaction without any protein purification steps, and immobilized antibodies demonstrated specific capture of their antigenic targets. We are now employing directed evolution approaches to improve surface display of scFv-intein fusion proteins, thereby increasing the production capacity of chemically-functionalized scFvs. Taken together, intein-linked yeast display may prove to be a particularly powerful tool for the rapid assessment of engineered proteins.