PFEnex

CASE STUDY

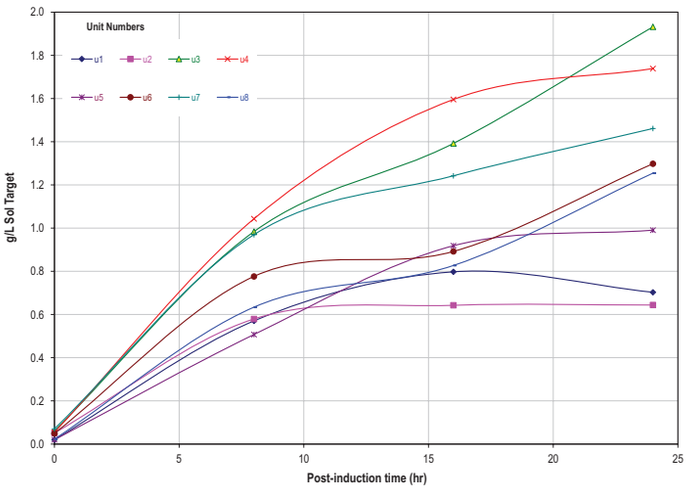
ANTIBODY EXPRESSION AND ENGINEERING FOR NON-STANDARD AMINO ACID INCORPORATION

Non-standard amino acid (NSAA) incorporation has many uses, including improved enzyme activity, increased protein stability, the introduction of new protein activities (e.g. new binding activities), as well as providing a target for site specific conjugation. Site specific modification of antibody fragments for conjugation of half-life extension moieties or small molecule drugs is an example of a well-known use for NSAA incorporation. Production of antibody fragments themselves can be challenging, let alone the additional complexity of NSAA incorporation.

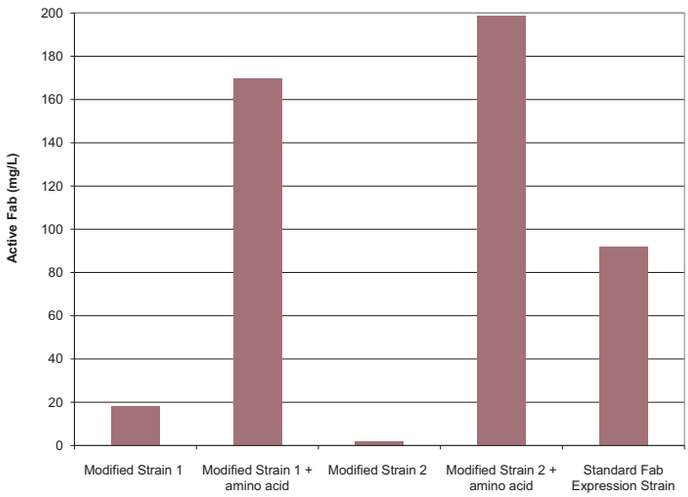
Following a strain engineering screen of several hundred strains, down-selected strains were scaled to 1L fermentations and assessed for robustness, with titers of up to ~2g/L of active Fab observed. Antigen binding was measured by biolayer interferometry, BLI.

To facilitate site specific pegylation, a subset of high producing strains were engineered to require incorporation of a NSAA for production of active Fab using Ambrx ReCODE™ technology. Incorporation of a nonstandard amino acid would then be used for site specific pegylation. *P. fluorescens* strains were engineered to express a modified Fab gene as well as ReCODE™ technology tRNA and tRNA synthetase genes. Fab expression was evaluated in the presence of the NSAA, and BLI was again used to measure active Fab yield (antigen binding). Similar titers were observed at small scale compared to "standard Fab" expression. Purified Fab was analyzed and incorporation of non-natural amino acid was confirmed by mass spectrometry. These data indicate that *P. fluorescens* is highly amenable to engineered NSAA incorporation without incurring reduced target protein titer.

Expression of "Standard Fab" at 1L scale under multiple induction conditions



Small Scale Expression of Modified Fab (without/with NSAA addition) compared to Standard Fab Expression



PFEnex

Pfenex, Inc. | 10790 Roselle Street | San Diego, CA 92121 | 858 352-4400 | www.pfenex.com

08/18 - Case Study: Antibody Expression and Engineering for Non-Standard Amino Acid Incorporation - 410-2c