## Genetic Code Expansion in *Bacillus subtilis*

Devon Stork[1]* (dstork@g.hmc.edu), Erkin Kuru,[1] Aditya M. Kunjapur,[1,2] Ethan Garner,[1] and **George Church**[1]

[1]Harvard University, Boston;[2]University of Delaware, Newark

**Our goals are to establish broad genetic code expansion tools in the primary gram-positive model bacterium *Bacillus subtilis*. We aim to transfer and characterize most noncanonical-amino acid incorporation systems present in *E. coli* to *B. subtilis* and utilize them for several applications.**

Encoding nonstandard amino acids (nsAAs) into proteins allows for expansion of the genetic code beyond the standard 20 amino acids for probing, labelling, or controlling proteins in a minimally disruptive manner. However, genetic code expansion has been unavailable in many bacterial model systems, such as the primary gram-positive model and common industrial organism, *Bacillus subtilis.* Here we describe the use of several classes of genome-integrated synthetases to incorporate a variety of different nsAAs into proteins in *B. subtilis* (figure 1) including nsAAs used for biorthogonal labelling, fluorescence imaging, and photo-crosslinking. We also demonstrate a nsAA-titratable protein expression system in this bacterium. Unlike *E. coli* codon expansion systems, where nsAAs were not incorporated into native UAG codons even before recoding efforts, *B. subtilis* nsAA systems incorporate nsAAs into many genomic proteins at native UAG codons. This feature presents both challenges and opportunities for follow-up work in *B. subtilis* nsAA research and genome modification. The general and effective expansion of nsAA technology to *B. subtilis* can facilitate new experiments in this important model bacterium and enable industrial protein production of nsAA-containing proteins.

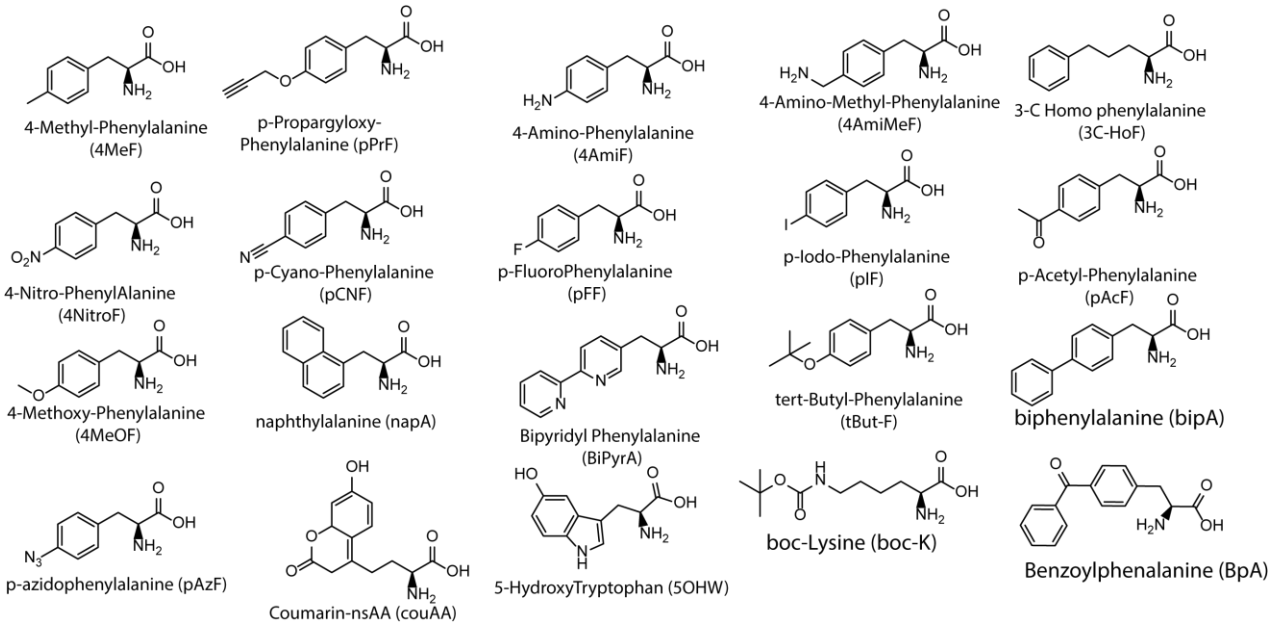


**Figure 1.** nsAAs so far incorporated into proteins in the organism *B. subtilis*

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