

Supporting Information:
Selective Dye-Labeling of Newly Synthesized Proteins in Bacterial Cells
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Plasmid and Expression Hosts. The plasmid pQE30-Barstar contains a gene encoding histidine-tagged barstar under control of a T5 promoter. Briefly, PCR was used to add *Bam*HI and *Hind* III sites to the barstar gene. The gene encodes two mutations (Cys53Ala, Cys95Ala) for improved stability that do not affect barnase binding¹. We do not believe that cysteine deletion is necessary for effective labeling; we have observed labeling of many cysteine-bearing proteins in companion experiments. After digestion, the gene was inserted into pQE30 between the *Bam*HI and *Hind*III restriction sites. For incorporation of homopropargylglycine (Hpg), pQE30-Barstar was transformed into the *E. coli* methionine auxotrophic strain M15-MA to make the expression host M15-MA [pQE30-Barstar].²

For incorporation of ethynylphenylalanine (Eth), pQE30-Barstar was linearized by digestion with *Nhe*I. The plasmid pQE15-PheRS* contains a mutant *E. coli* phenylalanyl-tRNA synthetase (A294G) under control of a modified *tac* promoter with an abolished *lac* repressor binding site for constitutive expression.^{3,4} This plasmid was digested with *Nhe*I, and a 1 kB fragment corresponding to the PheRS* cassette was isolated by agarose gel electrophoresis. This fragment was ligated into pQE30-Barstar to yield the plasmid pQE30-Barstar-PheRS*. This plasmid was transformed into the phenylalanine auxotrophic *E. coli* strain BL21(DE3) containing pLysS-IQ (AF-IQ) to make the expression host AF-IQ [pQE30-Barstar-PheRS*].⁴

Expression of Barstar. An overnight culture of M15-MA [pQE30-Barstar] was diluted 40-fold into 90 mL of M9 minimal medium containing all twenty natural amino acids (40 mg/L each; 20mg/L Phe for AF-IQ strain), ampicillin (200 mg/L), and kanamycin (35 mg/L). After reaching an OD₆₀₀ of 1, the culture was sedimented by centrifugation for 5 minutes (6500g) at 4 °C. The cell pellets were washed twice with NaCl (0.9 wt %). The culture was resuspended in M9 minimal medium without methionine. The culture was divided into 35, 48, and 5 mL aliquots. For protein synthesis inhibition, tetracycline (10 mg/mL) was added to the 5 mL culture. After 15 minutes at 37 °C, the samples were supplemented with either methionine (0.75 mM in 35 mL medium) or Hpg (1 mM in 48 mL medium). After 10 minute incubation, protein expression was induced for 3.5 h by the addition of IPTG (1 mM). The 5 mL culture supplemented with Hpg and tetracycline was not induced.

The expression of barstar from AF-IQ [pQE30-Barstar-PheRS*] was performed as above, except the M9 minimal medium was supplemented with chloramphenicol (35 mg/L) instead of kanamycin. After reaching an OD₆₀₀ of 1.0, a medium shift was performed as described. The cultures were supplemented with either phenylalanine (1.5 mM) or Eth (1.5 mM). Expression was induced for 3.5 h with IPTG (1 mM).

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- (2) Link, A. J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164-11165.
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- (4) Sharma, N.; Furter, R.; Kast, P.; Tirrell, D. A. *FEBS Lett.* **2000**, *467*, 37-40.