## Two-strain, cell-selective protein labeling in mixed bacterial cultures

Frank Truong<sup>1</sup>, Tae Hyeon Yoo<sup>2</sup>, Thomas J. Lampo<sup>†</sup>, and David A. Tirrell<sup>1,\*</sup>

## **Supporting Information**

	Connolly Solvent	Connolly Molecular
	Excluded Volume (Å <sup>3</sup> )	Area (Å <sup>2</sup> )
Methionine	126.964	154.321
Methionine Side Chain	94.736	124.784
Propargylglycine	93.655	120.317
Propargylglycine Side Chain	58.185	87.38

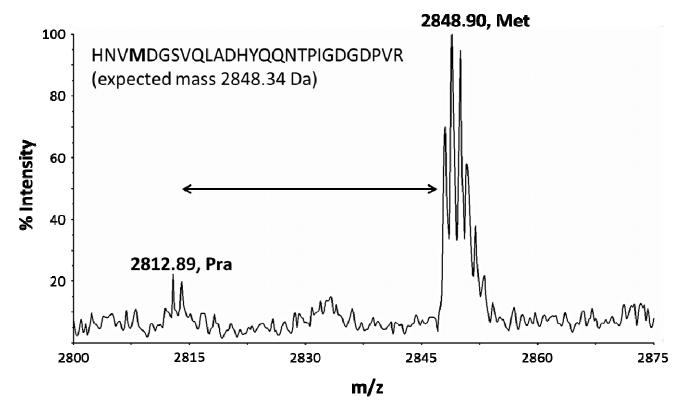
Table SI 1: Dimensions of methionine and propargylglycine. Values were calculated in

ChemBio3D Ultra.

<sup>&</sup>lt;sup>1</sup>Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, <sup>2</sup>Department of Molecular Science and Technology, Division of Applied Chemistry and Biological Engineering, Ajou University, Suwon, South Korea. \*Corresponding author: tirrell@caltech.edu

	Mutations	Normalized Fluorescence with Pra
MO2b_13-2	L13P, A256G, P257T, Y260Q, H301F	100%
Clone 1	<b>V328M</b> , V389L	143%
Clone 5	S207N, Q379H, Δ548W	151%
Clone 12	V328L	131%
Clone 15	<b>T339S</b> , I515S	136%
Clone 16	A331V	163%

**Table SI 2:** Selection of the best performing mutants from MO9c\_4 screened population compared to MO2b\_13-2. KMSKS motif is located at amino acids 332-336 in *E. coli* methionyl-tRNA synthetase. Mutations in bold are located in the flexible regions adjacent to the KMSKS sequence.



**Figure SI 1:** MALDI-TOF spectrum of tryptic digest of purified GFPrm\_AM. His-tagged protein was expressed in a Met-auxotrophic strain of *E. coli* harboring PraRS in medium supplemented with 20 amino acids (269 μM Met) plus 4 mM Pra. Replacement of Met by Pra in the peptide HNVMDGSVQLADHYQQNTPIGDGDPVR results in a decrease in mass of 36 Da.