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2 Cell-free Production of Integral Membrane Aspartic Acid Proteases Reveals Zinc-  
3 dependent Methyl Transferase Activity of the *Pseudomonas aeruginosa* Prepilin  
4 Peptidase PilD

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10 Supplementary Figure 1. SAM-dependent methylation of PilA is specific for Phe1.

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12 Chromatograms from N-terminal protein sequencing show SAM-dependent modification of the  
13 first residue, phenylalanine. Separate suspensions of PilD and PilA translation reactions were  
14 mixed together at final concentrations of 0.2% DDM, 100 mM HEPES-NaOH (pH 7.5), 1 mM  
15 DTT, 0.3 mg/mL PilD, and 0.9 mg/mL PilA (molar ratio of PilD:PilA=1:6.3) in the presence or  
16 absence of 80  $\mu$ M SAM, and incubated at 37° C for 2 h.

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18 A reference chromatogram of amino acid derivative standards (10 pmol each) is shown on the  
19 top, in which two peaks other than 20 amino acids, diphenylthiourea (dptu) and diphenylurea  
20 (dpu), are by-products of Edman degradation. N-methylphenylalanine (NmF) was detected with  
21 a retention time around 19 min, well-separated from the other amino acid derivatives, which is  
22 consistent to an increased hydrophobicity over phenylalanine (Strom *et al.*, 1993). The amount of  
23 each amino acid residue detected in each cycle is as follows: 31 pmol, F, residue 1 (control); 26  
24 pmol, T, residue 2 (control); 22 pmol, T, residue 2 (+SAM).

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26 Sequencing experiments were carried out at the Protein Facility of the Iowa State University. We  
27 are grateful to Joel Nott for assistance.

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29 Reference

30 Strom, M. S., D. N. Nunn & S. Lory, (1993) A single bifunctional enzyme, PilD, catalyzes  
31 cleavage and N-methylation of proteins belonging to the type IV pilin family. *Proc Natl*  
32 *Acad Sci U S A* **90**: 2404-2408.

