Supporting information for Moll *et al.* (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.232299399

Supporting Materials and Methods

Construction of Expression Vectors. Expression vectors were constructed by inserting PCR fragments of *streptavidin* (1) and *sbsB* (2) into the vector pET-28a(+) (Novagen). Template DNAs for PCR were the plasmid pUC8-SZ (kindly provided by Takeshi Sano, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, and Charles R. Cantor, Sequenom, San Diego, CA) for *streptavidin*, and chromosomal DNA of *G. stearothermophilus* PV72/p2 for *sbsB*. Biomass of *G. stearothermophilus* PV72/p2 was grown in continuous culture (3), and chromosomal DNA was prepared using a genomic DNA isolation kit (Qiagen). Restriction sites flanking the gene fragments were generated by using PCR primers with 5' extensions. All PCR fragments were amplified with proof-reading *Pwo* DNA polymerase (Roche Molecular Biochemicals). PCRs and cloning steps were performed according to standard procedures (4). Plasmids encoding four N-terminal and two C-terminal fusion proteins (Fig. 6) and a plasmid encoding minimum-sized core streptavidin (5) were cloned. For the construction of the four N-terminal fusion protein expression vectors, first an intermediate vector was made by using the primer pair SF1/SR1 (oligonucleotide primer sequences are listed below) to generate a *streptavidin* PCR fragment encoding amino acids 16-133 of the streptavidin sequence, and cloning it between the *Nco*I and *Eco*RI sites of pET-28a(+). In a second cloning step, pS1B1, which encodes streptavidin fused to position A1 of the SbsB protein sequence, was constructed by cloning an *sbsB*-fragment generated with the primer pair F5/R2 between the *Eco*RI and *Xho*I sites of the intermediate vector. Likewise, the plasmids pS1B-30, pS1B63 and pS1B177 encoding fusions of streptavidin to positions A-30 (in the signal peptide), T63 and E177 of SbsB were constructed using the primer pairs F4/R2, F7/R2 and F6/R2, respectively. The two C-terminal fusion protein expression vectors were made by first amplifying an *sbsB*-fragment, which encoded amino acids 1-889 of the SbsB protein, with the primer pair F2/R4 and cloning it between the *Nco*I and *Eco*RI sites of pET-28a(+). Subsequently, *streptavidin* fragments encoding amino acids 16-133 and 8-133 were amplified by using the primer pairs SF2/SR2 and

SF3/SR2 and cloned between the *Eco*RI and *Xho*I sites of pET-28a(+) to give the plasmids pBS1 and pBS2, respectively. The *Eco*RI site which was used to connect the two gene fragments translated to EF in all six vectors. Plasmid pS1 for expression of minimum-sized core streptavidin was constructed by amplifying a PCR fragment encoding amino acids 16-133 of the streptavidin sequence with the primer pair SF1/SR2. This fragment was cloned between the *Nco*I and *Xho*I sites of pET-28a(+). The following oligonucleotides were used as PCR primers (restriction sites are *italicized*, and start and reverse complementary stop codons are underlined):

F2: 5'-CGGAATT*CCATGG*CAAGCTTCACAGATGTTGC-3'

F4: 5'-CGGC*GAATTC*GCTTATCAACCTAAGTCCTATC-3'

F5: 5'-CGGC*GAATTC*GCAAGCTTCACAGATGTTGC-3'

F6: 5'-CGGC*GAATTC*GAAGTAACTGCGGTTAATTCG-3'

F7: 5'-CGGC*GAATTC*ACAGATGTGCCAAAAGAC-3'

R2: 5'-GACCG*CTCGAG*TTATTTTGTCACAGTCACATTGAC-3'

R4: 5'-CGGC*GAATTC*TTTTGTCACAGTCACATTGAC-3'

SF1: 5'-TACATG*CCATGG*GCATCACCGGCACCTG-3'

SR1: 5'-CGGC*GAATTC*CACCTTGGTGAAGGTGTCG-3'

SF2: 5'-CGGC*GAATTC*GGCATCACCGGCACCTG-3'

SR2: 5'-GACCG*CTCGAG*TTACACCTTGGTGAAGGTGTCG-3'

SF3: 5'-CGGC*GAATTC*GCCCAGGTCTCGGCCG-3'

Atomic Force Microscopy, Transmission Electron Microscopy and Digital Image Enhancement. Atomic force microscopy was performed by using a Digital Instruments Nanoscope IIIa. Recordings were made in contact mode under 100 mM NaCl by using a silicon nitride cantilever with a nominal spring constant of 0.06 N/m (NanoProbes, Digital Instruments). A Philips CM12 TEM/STEM at 80 kV was used for transmission electron microscopy. Ultrathin sectioning and negative staining were carried out as described (6). Electron micrographs for digital image enhancement were recorded with typical defocus values of 300-500 nm. Digitized micrographs (512 by 512 pixels) were Fourier processed, as described (7).

1. Argaraña, C. E., Kuntz, I. D., Birken, S., Axel, R. & Cantor, C. R. (1986) *Nucleic Acids Res.* **14,** 1871–1882.

2. Kuen, B., Koch, A., Asenbauer, E., Sára, M. & Lubitz, W. (1997) *J. Bacteriol.* **179,** 1664–1670.

3. Sára, M., Kuen, B., Mayer, H. F., Mandl, F., Schuster, K. C. & Sleytr, U. B. (1996) *J. Bacteriol.* **178,** 2108–2117.

4. Sambrook, J. & Russell, D. W. (2001) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).

5. Sano, T., Pandori, M. W., Chen, X., Smith, C. L. & Cantor, C. R. (1995) *J. Biol. Chem.* **270,** 28204–28209.

6. Messner, P., Hollaus, F. & Sleytr, U. B. (1984) *Int. J. Syst. Bacteriol.* **34,** 202–210.

7. Sleytr, U. B., Messner, P. & Pum, D. (1988) in *Methods in Microbiology*, ed. Mayer, F. (Academic, London), Vol. 20, pp. 29–60.