Supporting Information

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SI Materials and Methods

Plasmid and Strain Constructions. The *MS-DesK* gene and its variants were PCR-amplified from plasmid TM1/5-DesKC-pHPKS or from DesK-pHPKS for full-length DesK (1, 2). Site-directed mutagenesis was performed to introduce the mutations A3, Q3, SB+/Q3, and SB^{-}/A3 using different pairs of mutagenic oligonucleotides. The overlap-PCR products were cloned into the BamHI-XbaI sites of the expression vector pHPKS, a low copy number plasmid that places the coding regions under the control of the P*Xyl* promoter (2). The resulting plasmids were used to transform the *desK*−CM21 or AKP20 *Bacillus subtilis* cells (1, 3, 4). To induce the expression of DesK variants, 0.1% xylose was added to the growth medium. To overexpress and purify DesK variants for in vitro assays, the same amplicons were cloned into the NdeI-SalI sites of expression vector pET22 (Novagen), and the resulting plasmids were used to transform BL21 cells. All mutations were confirmed by DNA sequence analysis. Strains, full sequences, and detailed construction methods are available upon request.

Bacterial Strains and Growth Conditions. *B. subtilis* JH642 strains were grown in a rotary shaker (New Brunswick, model VS) operating at 250 rpm. Cells were either grown at 37 °C, or at 37 °C and then transferred to 25 °C at an $OD_{525} = 0.3$ in Spizizen salts supplemented with 0.1% glycerol, 50 μg/mL each tryptophan and phenylalanine, 0.05% casa amino acids, and trace elements (5, 6). β-galactosidase activity was assayed in independent triplicates (7). The results shown are the average of three independent experiments and correspond to 2 h after the cold shock.

Turbidity Measurements. Right-angle light scattering was measured in an SLM-8000C spectrofluorimeter with 400 nm as excitation and emission wavelengths. The sample was maintained under continuous stirring with a small magnetic bar in the measurement cuvette. Two microliters of peptide dissolved in water at 10 mg/mL were added at 50 and 100 s to a 0.1 mg/mL liposome suspension in 10 mM Hepes-Na buffer at pH 7.3 in a final volume of 1 mL. When required, KCl was added to the liposome suspension before peptide addition.

Protein Overexpression, Purification, and Reconstitution. The *Escherichia coli* BL21 strain was used as a host for plasmid pET 22b, which overexpressed MS-DesK and its variants. The overexpression and purification of this protein was performed following the Studier Method for Autoinduction of Protein Expression in the T7 System (8). Cells were resuspended in TNP buffer [50 mM Tris (pH 8), 200 mM NaCl, 1 mM phenylmethylsulfonyl fluoride) supplemented with 20 mM imidazole (TNPI-20) and a mixture of Triton X-100 and Brij 58, both at 0.5%. After treatment with lysozyme 1 mg/mL for 30 min, cells were disrupted by sonication and then centrifuged at 37,000 × *g* for 15 min. From the supernatant, MS-DesK in mixed micelles was purified by affinity chromatography using an Ni^{2+}-nitrilotriacetic acid agarose resin (Qiagen) at 4 °C for 1 h and dialyzed against 50 mM Tris (pH 8), 200 mM NaCl, 10% (vol/vol) glycerol, and 1 mM DTT.

Proteoliposome Preparation. Eight milligrams of lipids of *E. coli* polar lipids extract or 1,2-dimyristoyl-sn-glycero-3-phosphocholine lipids (Avanti Polar Lipids) were hydrated in 1 mL of hydration buffer [20 mM Tris·HCl (pH 8), 250 mM sucrose, and 100 mM K_2SO_4) and vortexed above the melting temperature. The resulting large multilamellar vesicle suspensions were disrupted by 10 freeze–thaw cycles. For liposome generation, lipid suspensions were extruded through a 200-nm pore size Whatman polycarbonate filter using a hand-held extrusion device (Avanti). A homogenous suspension of unilamellar liposomes was obtained. To insert the protein in liposomes, 0.24% (wt/wt) Triton X-100 was added to preformed liposomes and incubated for 20 min with continuous stirring. Then, purified MS-DesK was added at an 80/1 (wt/wt) lipid/protein ratio and incubated with continuous stirring during 1 h at 4 °C. The detergent was later removed by incubating the sample 8 h with SM2 Bio-beads (Biorad). This detergent-removal step was repeated three times, shifting the equilibrium toward the integration of the protein into the liposomes.

Proteoliposome-Purification Step. MS-DesK–containing proteoliposomes were separated from free liposomes and not-incorporated protein on a sucrose gradient by ultracentrifugation. The liposome suspension collected after the Bio-Beads treatment was placed on the bottom of a step sucrose gradient (1.6, 1.2, and 0.2 M sucrose) and centrifuged overnight at 30,000 rpm in an SW40 rotor at 4 °C. After centrifugation, the proteoliposome band floating on top of the gradient was harvested, washed with 30 mM Tris·HCl (pH 8.0) and ultracentrifuged in a Ti90 rotor at 45,000 rpm for 1 h at 4 °C. Proteoliposomes containing MS-DesK were resuspended in 200 μL of hydration buffer, glycerol 10%, and DTT 1 mM. The protein concentration was determined by Lowry, and the quality of the sample, as well as the efficiency of protein integration, was determined by SDS/PAGE followed by Western blot using a-His antibodies (Qiagen) and a-DesKC antibodies.

1. Albanesi D, Mansilla MC, de Mendoza D (2004) The membrane fluidity sensor DesK of *Bacillus subtilis* controls the signal decay of its cognate response regulator. *J Bacteriol* 186(9):2655–2663.
2. Cybulski LE, Martín M, Mansilla MC, Fernández A, de Mendoza D (2010) Membrane thickness cue for cold sensing in a bacterium. *Curr Biol* 20(17):1539–1544.
3. Aguilar PS, Hernandez-Arriaga AM, Cybulski LE, Erazo AC, de Mendoza D (2001) Molecular basis of thermosensing: A two-component signal transduction thermometer in *Bacillus subtilis*. *EMBO J* 20(7):1681–1691.
4. Johansson P, Hederstedt L (1999) Organization of genes for tetrapyrrole biosynthesis in gram-positive bacteria. *Microbiology* 145(Pt 3):529–538.
5. Spizizen J (1958) Transformation of biochemically deficient strains of bacillus subtilis by deoxyribonucleate. *Proc Natl Acad Sci USA* 44(10):1072–1078.
6. Harwood CR, Cuttings SM, eds (1990) *Molecular Biological Methods for Bacillus* (Wiley, Chichester, England).
7. Miller JH (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY).
8. Studier FW, Moffatt BA (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J Mol Biol* 189(1):113–130.

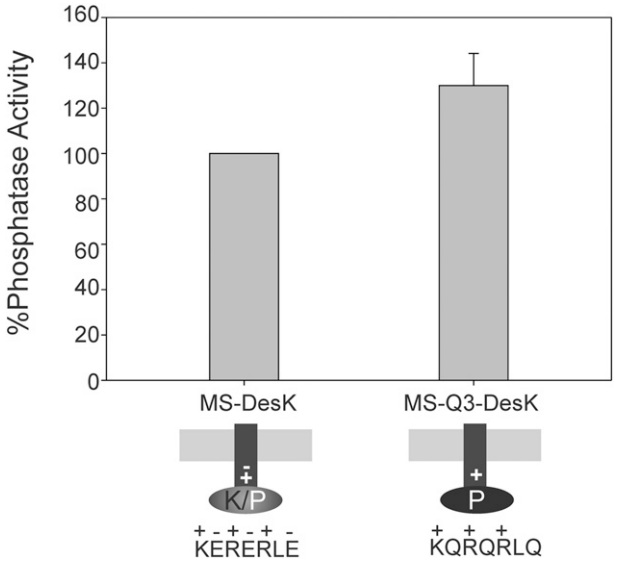

Fig. S1. MS-Q3-DesK conserves phosphatase activity. In vivo phosphatase assay was performed using strain AKP20, which contains a P*des-lacZ* construct and overexpresses phospho-DesR, in a desK−null background. This strain shows constitutive expression of the reporter gene β-galactosidase at 37 °C unless it is complemented with DesK variants exhibiting phosphatase activity (2, 3). AKP20 cells complemented with pHPKS plasmids carrying MS-DesK with the WT or Q3 linker variants were grown at 37 °C in the presence or absence of the inductor (xylose), and β-galactosidase activities were determined every hour. The difference in β-galactosidase activity in the absence or presence of the inductor reflects the phosphatase activity of each variant. A 100% difference corresponds to the MS-DesK with a WT linker.

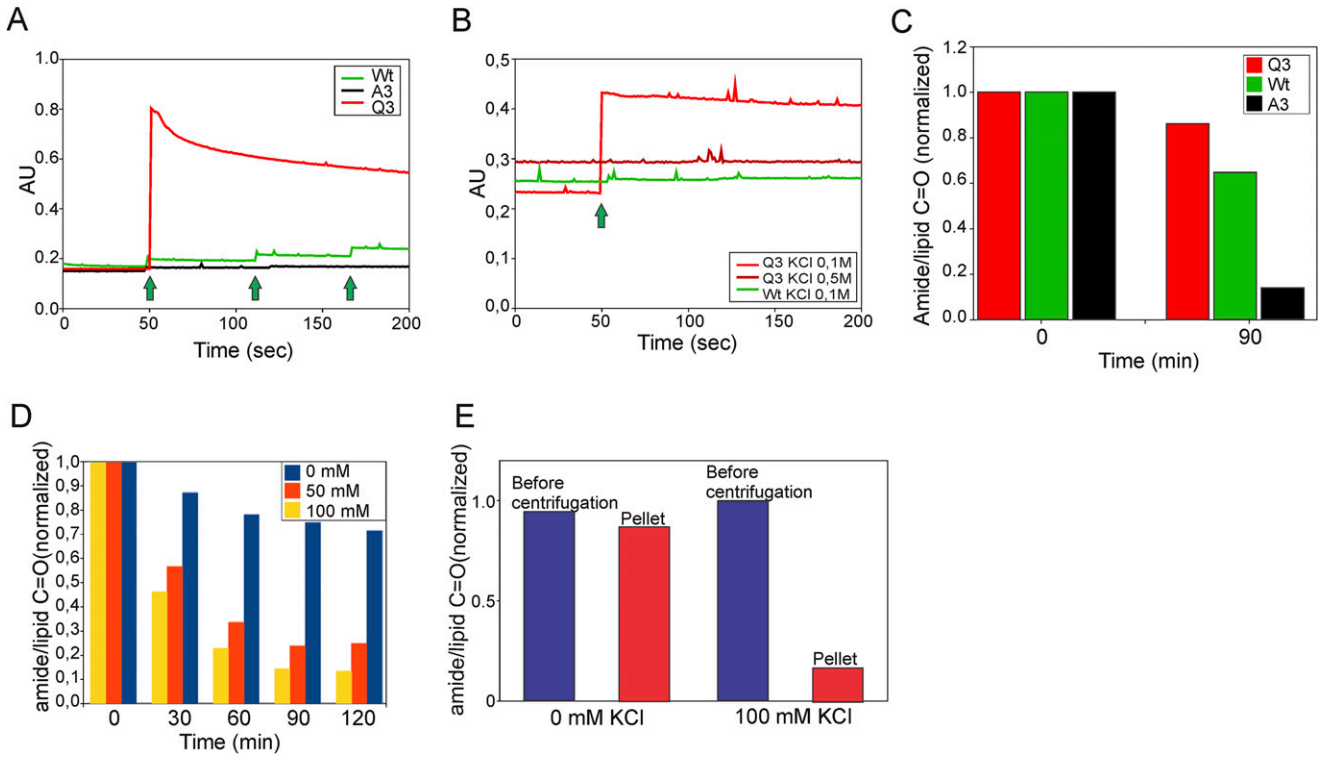

Fig. S2. Peptide–lipid interaction. Turbidity of liposomal suspensions upon sequential addition of peptides (arrows) measured by right-angle light scattering in the absence (*A*) or presence (*B*) of KCl. Attenuated total reflection (ATR)-FTIR analysis of peptide–liposome complexes subjected to different conditions: microdialysis treatment (*C*); increasing salt concentrations followed by microdialysis for the indicated time periods (*D*), or followed by ultracentrifugation (*E*). Quantification of bound peptide is expressed as the ratio of the integrated amide I band of the peptide between 1,600 cm^{-1} and 1,700 cm^{-1} over the integrated C = O band at 1,735cm^{-1} of the lipids.

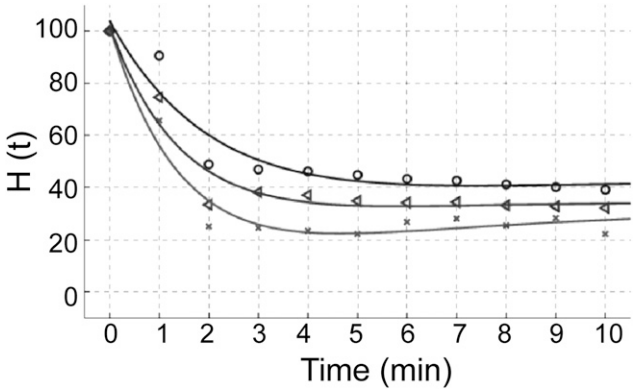

Fig. S3. Evolution of the percentage of exchange of the hydrogen of the peptidic amide group as a function of deuteration time. Peptides were mixed with *E. coli* polar lipid liposomes, and mixtures were flushed continuously with D2O-saturated nitrogen on the ATR element. Spectra were recorded every minute (32 scans averaged). The area of the amide II band (around 1,545 cm^{-1}), which is sensitive to the deuteration of the amide N-H group, was monitored over time and normalized on the lipid ester C = O group area (around 1,735 cm^{-1}). Circle, A3 peptide; triangle, WT peptide; cross, Q3 peptide.

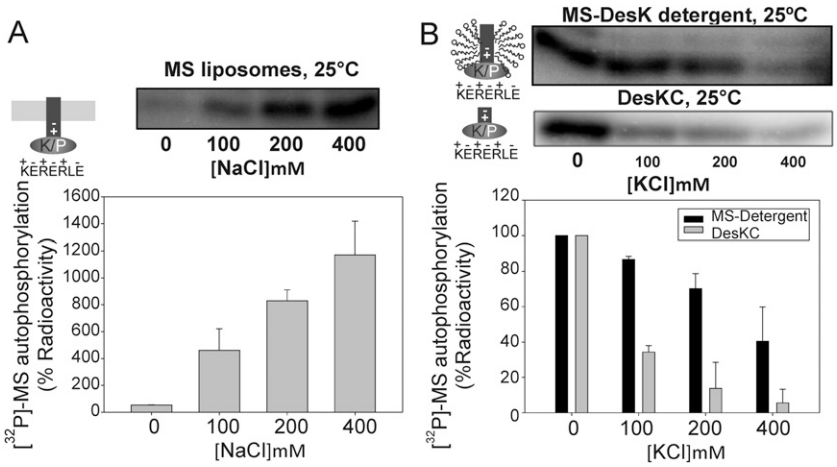

Fig. S4. Salt effect on autokinase activity of DesK variants. (*A*) The MS-DesK was reconstituted in liposomes of *E. coli* polar lipids, and autokinase activity (shown in *Upper*) was assayed at 25 °C in the presence of γ-32 ATP and increasing concentrations of NaCl. (*B*) The soluble truncated DesKC (gray bars) or the MS-DesK dissolved in Triton X-100 0.5%/Brij 0.5% (black bars) were incubated with increasing salt concentrations, and the autokinase activity was determined as in *A*. The total amounts of phosphorylated protein present in each well were determined by densitometry and plotted as the percentage of activity, considering 100% for the activity in the absence of salts (lane 1). The results correspond to the average of at least three independent experiments.

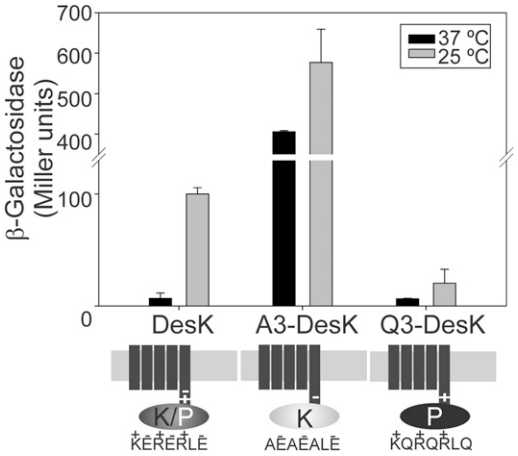

Fig. S5. CM21 cells expressing full-length DesK linker variants were grown at 37 °C or at 25 °C, and β-galactosidase–specific activities were determined every hour as described for Fig. 2. The results shown are the average of three independent experiments made 2 h after the cold shock.