Figure S1

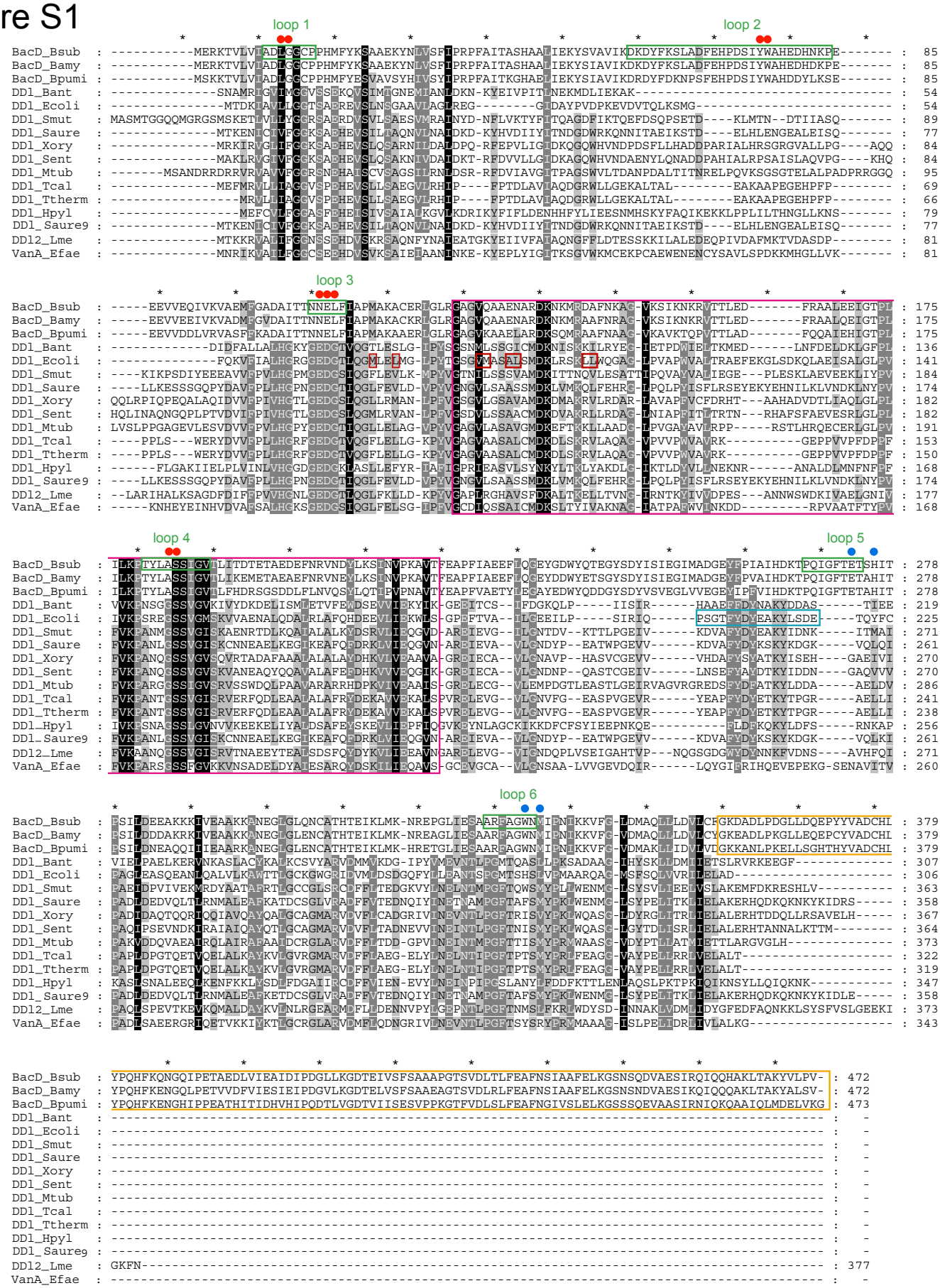

Figure S1. Amino acid sequence alignment of BacD and its homologues. The ω-loop and the residues involved in dimerization of DdlB from *E. coli* are enclosed with cyan and brown rectangles, respectively. The consensus ATP-grasp domains and the BacD-specific insertions are shown with the pink and yellow rectangles, respectively. Six loops interacting with amino-acid substrates in BacD are indicated and labeled with green. The residues around the side chains of the N-terminal and C-terminal amino-acid substrates, are marked with the blue and red circles, respectively. The sequences (and gene/PDB ID) shown are BacD from *Bacillus subtilis* (BacD_Bsub: NP_391651), BacD from *Bacillus amyloliquefacien* DSM 7 (BacD_Bamy: CBI_44730), BacD from *Bacillus pumilus* ATCC 7061 (BacD_Bpumi: ZP_03055035), Ddl from *Bacillus anthracis* (DDl_Bant: 3R23), Ddl from *E. coli* (DDl_Ecoli: 2DLN), Ddl from *Streptococcus mutans* (DDl_Smut: 3K3P), Ddl from *Staphylococcus aureus* (DDl_Saure: 2I87), Ddl from *Xanthomonas oryzae* pv. oryzae KACC10331 (DDl_Xory: 3E5N), Ddl from *Salmonella enterica* (DDl_Sent: 3I12), Ddl from *Mycobacterium tuberculosis* (DDl_Mtub: 3LWB), Ddl from *Thermus caldophilus* (DDl_Tcal: 2FB9), Ddl from *Thermus thermophilus* HB8 (DDl_Ttherm: 2YZG), Ddl from *Helicobacter pylori* (DDl_Hpyl: 2PVP), Ddl from *Staphylococcus aureus* (VRSA-9) (DDl_Saure9: 3N8D), Ddl from *Leuconostoc mesenteroides* (DDl2_Lme: 1EHI), and VanA from *Enterococcus faecium* BM4147 (VanA_Efae: 1E4E).

Figure S2

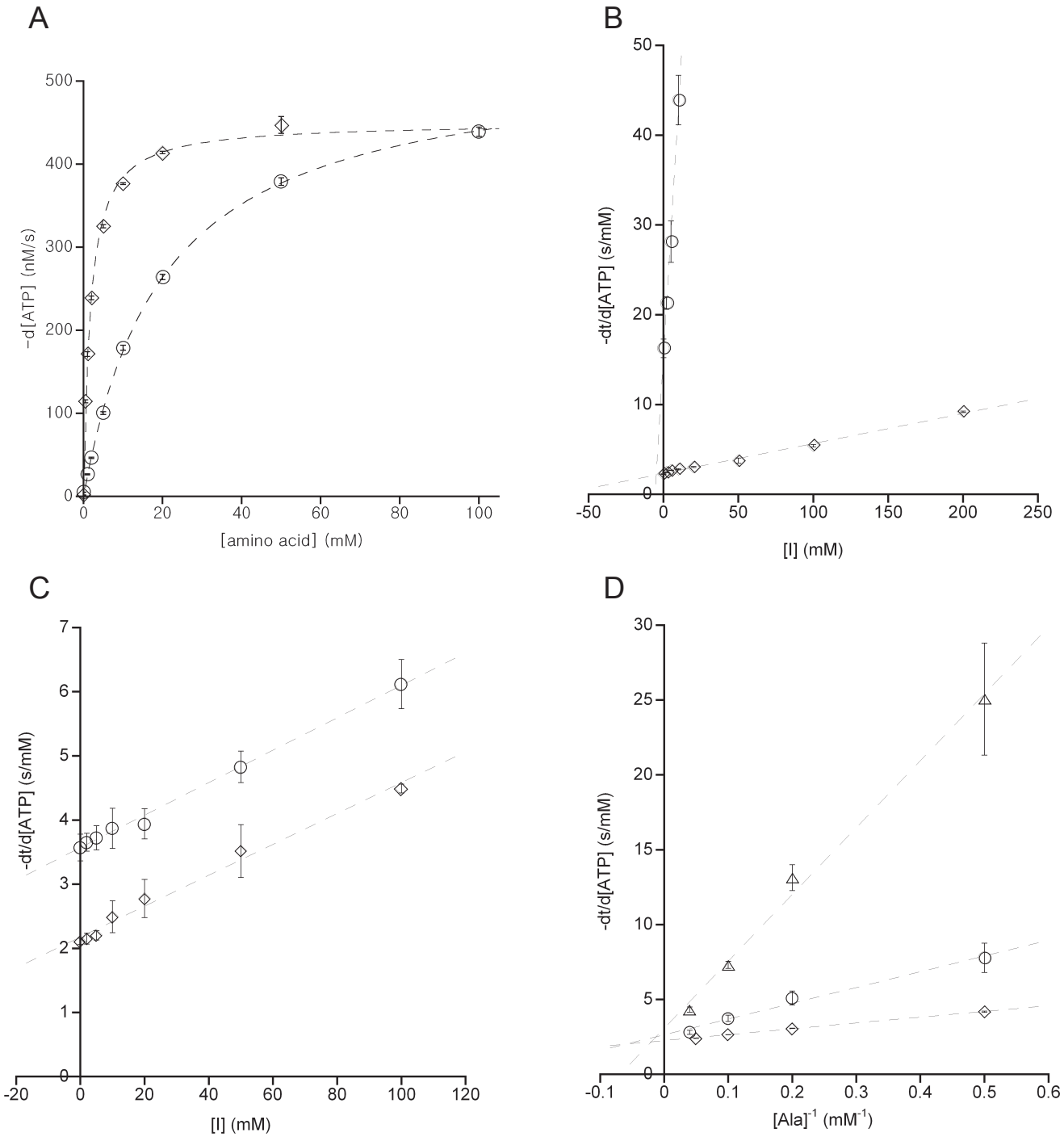

Figure S2. Enzymatic characterization of BacD. A: Rates of ATP hydrolysis were plotted against concentration of L-alanine (open square) and L-phenylalanine (open circle), where 100 mM L-phenylalanine and 25 mM L-alanine were included, respectively. Measurements were repeated at least three times and the standard deviations are indicated with error bars. Dashed lines show curve fittings with the Michaelis-Menten equation. B: Dixon plots are shown for the measurements in a range of concentrations of the phosphinate L-alanyl-L-phenylalanine analog, where 2 mM L-alanine and 100 mM L-phenylalanine (open circle) or 25 mM L-alanine and 100 mM L-phenylalanine (open square) were included. C: Dixon plots are shown as in B, but 25 mM L-alanine and 20 mM L-phenylalanine (open circle) or 25 mM L-alanine and 100 mM L-phenylalanine (open square) were included. D: Lineweaver-Burk plot is shown in a range of concentrations of L-alanine, where 100 mM L-phenylalanine and 0 mM (open square), 20 mM (open circle), or 100 mM (open triangle) of the phosphinate L-alanyl-L-phenylalanine analog were included.

Figure S3

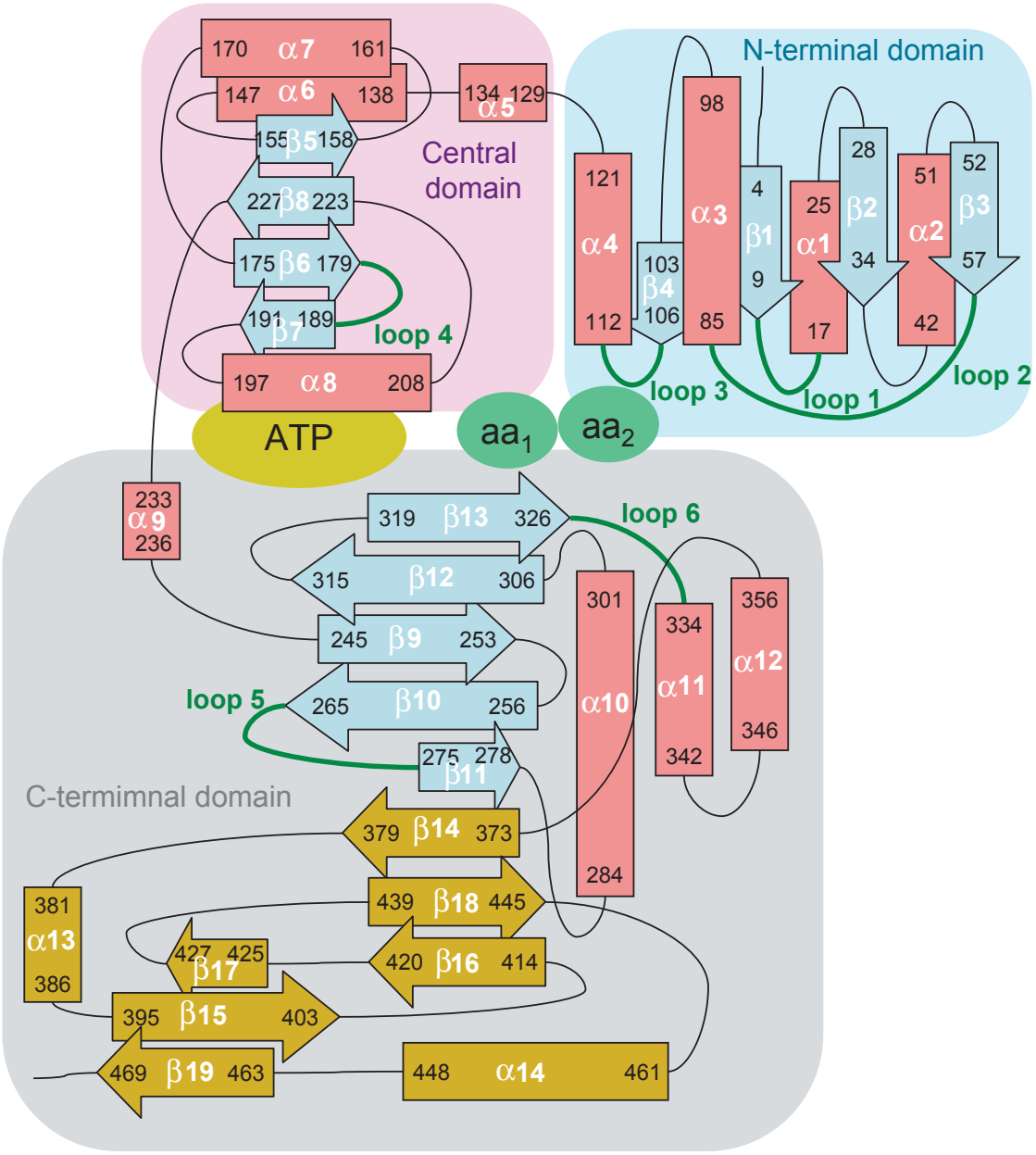

Figure S3. Topology diagram of BacD. α-helices (α1-α14) and β-strands (β1-β19) are shown in rectangles and arrows, respectively. The residue of start and end in each element are numbered. The approximate positions for ATP, first amino acid (aa_1), and second amino acid (aa_2) are indicated. The loops involved in the formation of the amino-acid bindig site are labeled "loop 1-6". The BacD-specific region at the C-terminal domain is shown with yellow.

Table S1. Primer sequences used for cloning and site-directed mutagenesis

Primer	Sequence 5′–3′
Cloning	
Forward with NcoI site	TAATACACCATGGAGAGAAAAACAGTATTGG
Reverse with XhoI site	AATACTCGAGTCATACTGGCAGCACATACTTTGCC
Mutagenesis	
Tyr75Phe-1	ATTCCATTTTTTGGGCGCATGAAGATCATAACAAGCCTGAGG
Tyr75Phe-2	TGCGCCCAAAAAATGGAATCAGGGTGTTCAAAATCAGCTAAAC
Tyr75Phe-3	TGAAGATCATAACAAGCCTGAGG
Tyr75Phe-4	CAGGGTGTTCAAAATCAGCTAAAC
Ser185Ala-1	ACTTAGCGGCTTCTATCGGTGTAACGCTGATTACGGACACTG
Ser185Ala-2	CGATAGAAGCCGCTAAGTATGTAGGCTTTAAGATAAGAGGTG
Ser185Ala-3	GTGTAACGCTGATTACGGACACTG
Ser185Ala-4	ATGTAGGCTTTAAGATAAGAGGTG