

## Supplementary Material

# **Structural Analysis of Spermidine Synthase from *Kluyveromyces lactis***

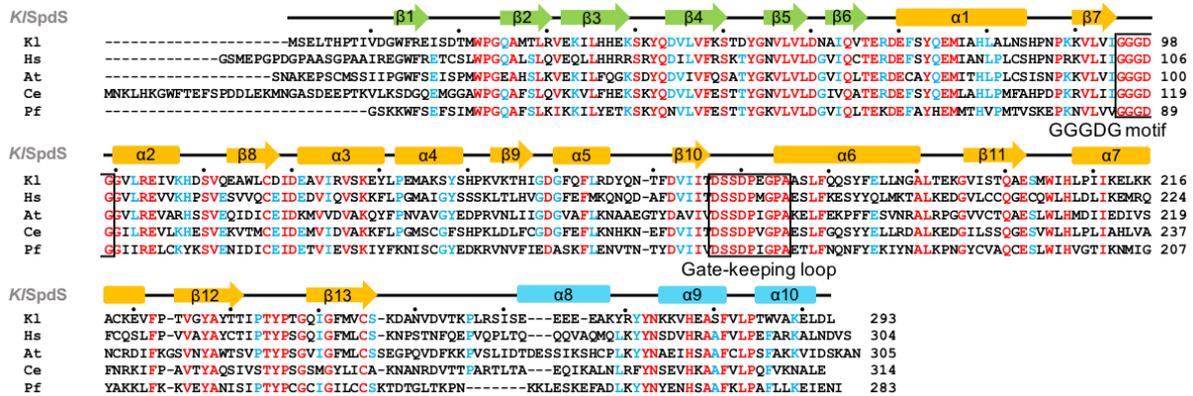
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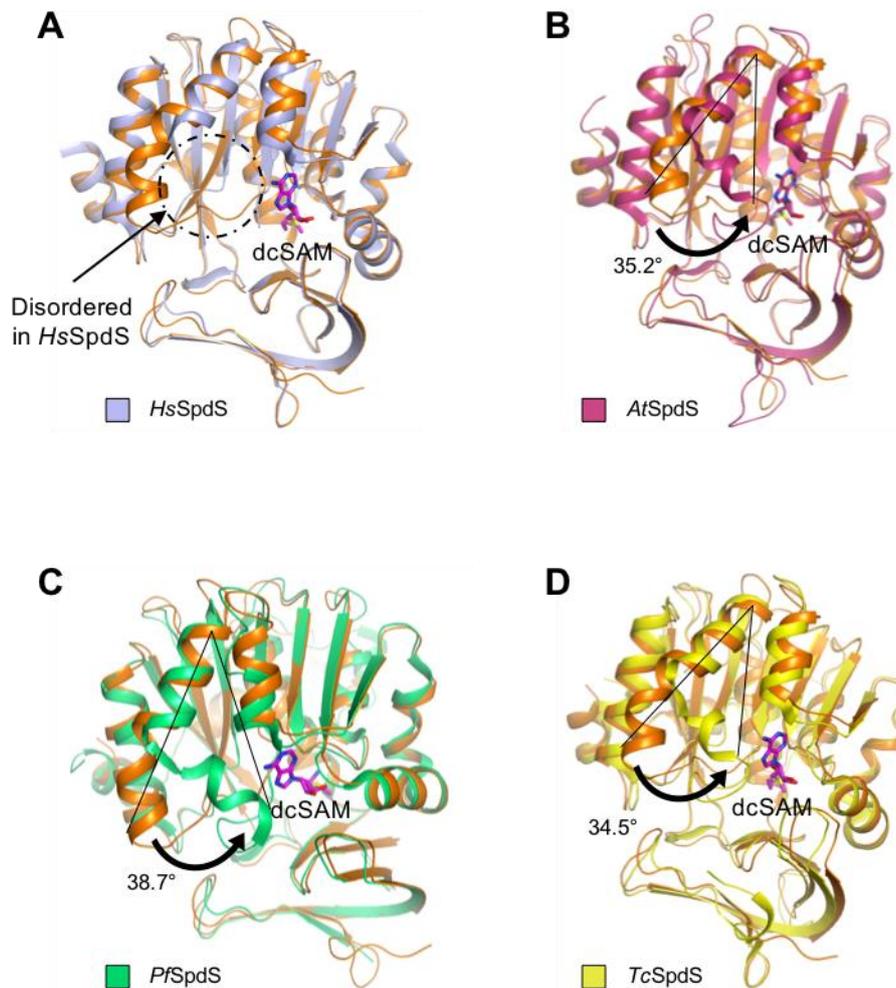
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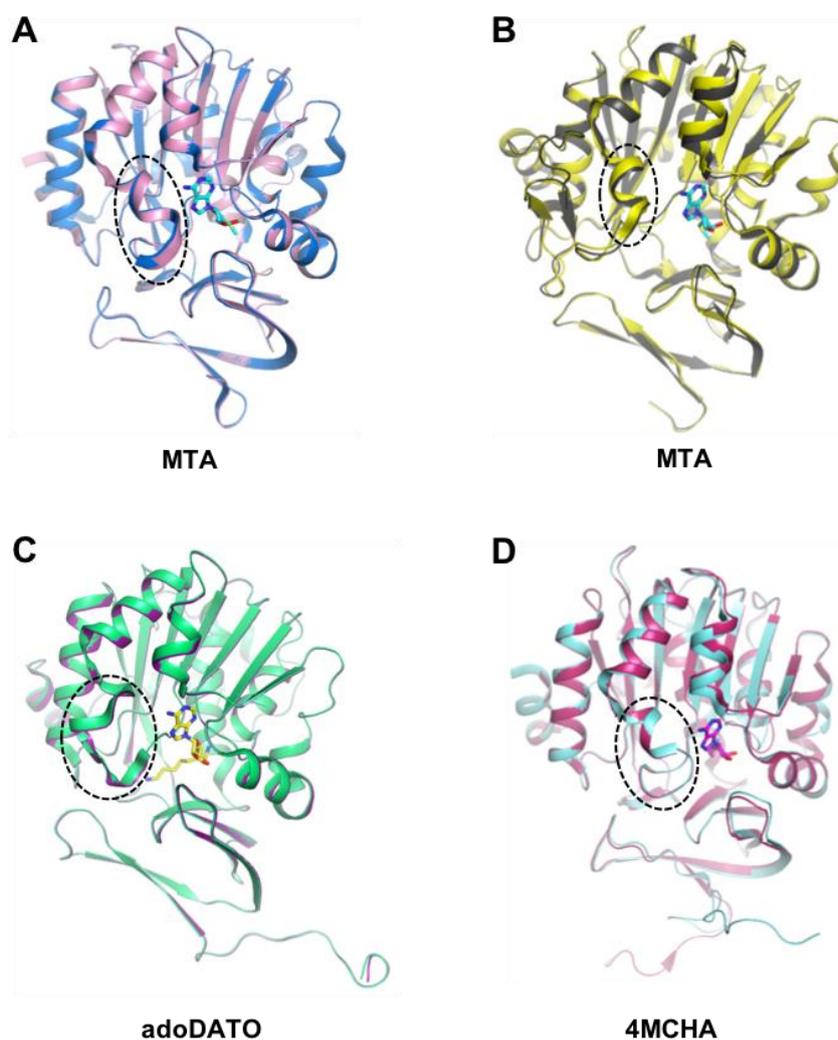
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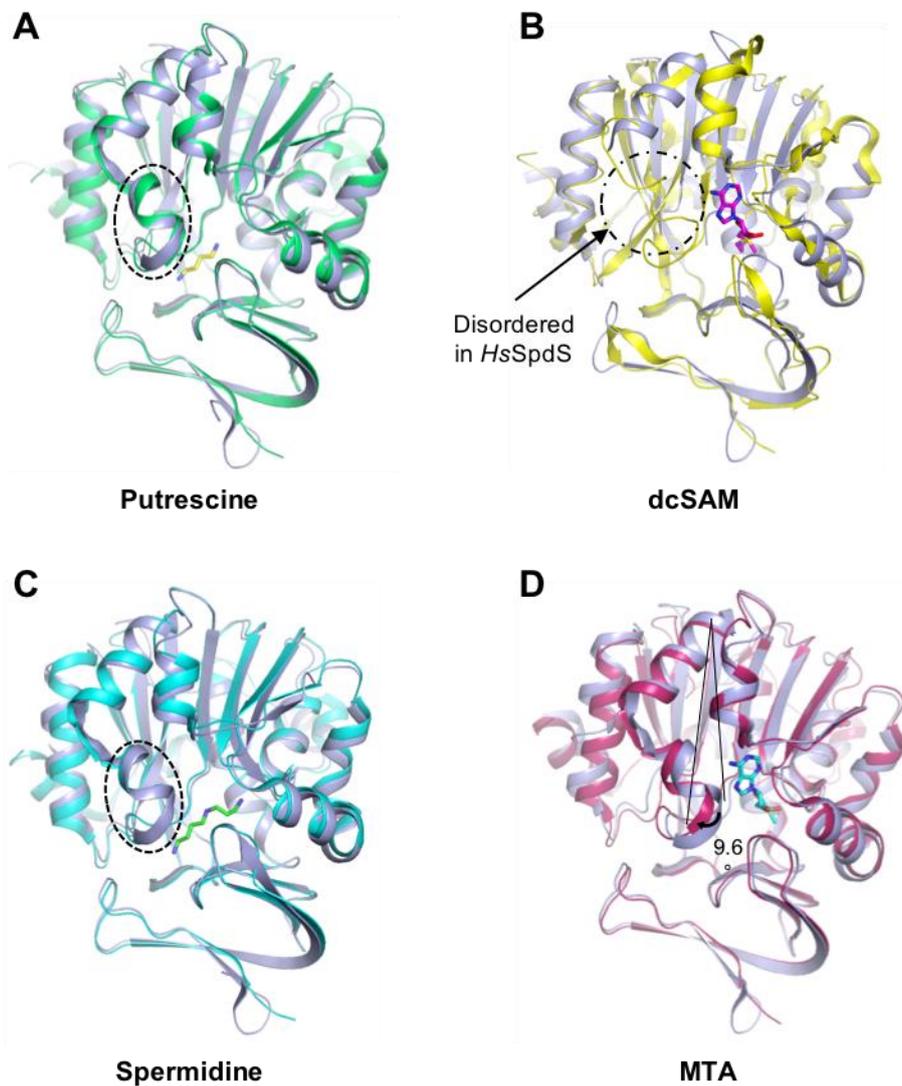
**Supplemental Figure S1. Structure-based amino acid sequence alignment of spermidine synthase (SpdS) from *Kluyveromyces lactis* (K1SpdS) with other homologs.** Residues with 100% identity are shown in red. Residues with 80% identity are shown in cyan. Black stars indicate three critical aspartic residues (Asp 98, Asp 167, and Asp 170 in K1SpdS) involved in the aminopropyltransferase reaction. Abbreviations: *Hs*: *Homo sapiens*. *At*: *Arabidopsis thaliana*. *Ce*: *Caenorhabditis elegans*. *Pf*: *Plasmodium falciparum*.



**Supplemental Figure S2. Comparison of the gate-keeping loop between the apo structures of spermidine synthase (SpdS) from *Kluyveromyces lactis* (*KlSpdS*) and SpdS complex structures from four other species.** Each figure represents monomer superposition to compare the gate-keeping loop. In (A)–(D), the monomer of apo-*KlSpdS* is shown in orange. (A) Superposition of apo-*KlSpdS* and the SpdS complex structures from *Homo sapiens* (*HsSpdS*; PDB code 2O06). The monomer of apo-*HsSpdS* is shown in light blue. (B) Superposition of apo-*KlSpdS* and the SpdS complex structures from *Arabidopsis thaliana* (*AtSpdS*; PDB code 6O65). The monomer of apo-*AtSpdS* is shown in warm pink. (C) Superposition of apo-*KlSpdS* and the SpdS complex structures from *Plasmodium falciparum* (*PfSpdS*; PDB code 2HTE). The monomer of apo-*PfSpdS* is shown in lime green. (D) Superposition of apo-*KlSpdS* and the SpdS complex structures from *Trypanosoma cruzi* (*TcSpdS*; PDB code 4YUV). The monomer of apo-*TcSpdS* is shown in yellow.



**Supplemental Figure S3. Comparisons of the gate-keeping loops between apo structures of spermidine synthase (SpdS) and SpdS-substrate complex structures from four species.** (A) Superposition of apo-SpdS (PDB code 2O0L) and the SpdS (PDB code 2O05) complex in *Homo sapiens*. The apo monomer is shown in marine and that of the complex structure is shown in pink. (B) Superposition of apo-SpdS (PDB code 1UIR) and the complex structure of SpdS (PDB code 3ANX) in *Thermus thermophilus*. The apo monomer is shown in yellow and that of the complex structure is shown in dark gray. (C) Superposition of the apo-SpdS (PDB code 1INL) and the complex structure of SpdS (PDB code 1JQ3) in *Thermotoga maritima*. The apo monomer is shown in lime green and that of the complex structure is shown in purple. (D) Superposition of apo-SpdS (PDB code 6O63) and the complex structure of SpdS (PDB code 6O65) in *Arabidopsis thaliana*. The apo monomer is shown in warm pink and that of the complex structure is shown in aquamarine.



**Supplemental Figure S4. Comparison of the gate-keeping loop in spermidine synthase (SpdS) complex structures from different species.** In (A) to (D), the monomer of the *HsSpdS* complex structure is shown in light blue. (A) Superposition of putrescine complex of *HsSpdS* (PDB code 2O06) and *PfSpdS* (PDB code 2HTE). The monomer of the *PfSpdS* complex is shown in lime green. (B) Superposition of the dcSAM complex of *AtSpdS* (PDB code 6O65) and *PfSpdS* (PDB code 2PT6). The monomer of the *PfSpdS* complex is shown in yellow. (C) Superposition of the spermidine complex of *HsSpdS* (PDB code 2O07) and *PfSpdS* (PDB code 2PWP). The monomer of the *PfSpdS* complex is shown in cyan. (D) Superposition of the MTA complex of *HsSpdS* (PDB code 2O05) and *PfSpdS* (PDB code 4BP1). The monomer of the *PfSpdS* complex is shown in warm pink.