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Supporting Information

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Self-Resistance to an Antitumor Antibiotic: A DNA Glycosylase Triggers the Base-Excision Repair System in Yatakemycin Biosynthesis**

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Supporting Information:

Materials and Methods

General. A YTM producer, *Streptomyces* sp. TP-A0356, was cultured as described.[7] *Escherichia coli* DH5α competent cells were used for routine subcloning and plasmid preparations. *E. coli* cells were grown in LB medium with appropriate antibiotics, when necessary. PCR amplification was carried out using PfuUltra^{TM} DNA polymerase with fosmid pTG1301 as a template. Primer synthesis was performed at Invitrogen Shanghai Center. DNA sequencing was performed at the Shanghai GeneCore Biotechnology Inc. All other common biochemicals and chemicals were from standard commercial sources. The genetic manipulations of *Streptomyces*, fermentation, and production analysis were all carried out as described previously.[10]

Construction of Gene Replacement Mutant and Complementation Mutants. To delete the *ytkR2* gene, a 2.4-kb *Hin*dIII/*Xba*I PCR fragment (5'-TAAAAGCTTGGACCGACGCGAATACGC and 5'-TAATCTAGAGCCTACCGGC-ACGGAGAC as primers) and a 2.1-kb *Xba*I/*Eco*RI fragment (5'-TAATCTAGATGTCGTCTCGGCGTCCAC and 5'-TAAGAATTCGACATGGCCGATCTGACG as PCR primers) were successively ligated and cloned into *Hin*dIII/*Eco*RI sites of pKC1139 to yield pTG1311. The result plasmid pTG1311 was introduced into *Streptomyces* sp. TP-A0356 by conjugation from *E. coli* S17-1 and colonies with apramycin (50 μg/mL) resistance were identified as single-crossover mutants. Further incubation and screening for colonies that were apramycin-sensitive and could give a 1.3-kb PCR fragment (Figure S1, 5'-GAGAAGGCCACCATCTTC and 5'-GGACATCCTCGGTATCGC as PCR primers) were selected as double-crossover mutant strain TG1308 in which the *ytkR2* gene was knocked-out by in-frame deletion.

To express *ytkR2* gene *in trans*, a 0.9-kb *Eco*RI/*Nde*I fragment (amplified with primers 5'-TAAGAATTCGTGTC-AGGGCGGGCTCTC and 5'-TAACATATGCGATCCCCGACGCGCGCG) containing the intact *ytkT* gene was cloned into pSET152 vector to yield plasmid pTG1312. It was introduced into *Streptomyces* sp. TP-A0356 by conjugation to produce complemented strain TG1309 and the fermentation broth was analyzed by HPLC for YTM production as described before with the wild-type strain as a control.[10]

Cloning and Protein Overproduction. *ytkR2* gene was amplified from cosmid pTG13001 ligated into the *Nde*I and *Xho*I sites of expression vector pET37b, to produce plasmid pTG1313. The *alkC*, *alkD* genes were amplified from plasmid pT7SCII-AlkC and pT7SCII-AlkD, respectively, and ligated into the *Bam*HI and *Eco*RI sites of expression vector pRSET B. The expression plasmid with *ytkR2* or its mutants was transformed into *E. coli* BL21 (DE3). The transformants were grown in LB medium (500 mL) containing kanamycin (25 mg/mL) at 37℃ to an OD_{600} of 0.5~0.6 and then induced with 0.1 mM isopropyl β-D-thiogalactoside and grown for 8 hours at 30℃. The AlkC and AlkD were overexpressed as described previously.[13] The His-tagged YtkR2, AlkC, AlkD and the mutant proteins were purified with Ni-IDA agarose column and buffer-exchanged to 50 mM Tris-HCl (pH=7.5), 50 mM NaCl and 10% glycerol. Protein concentrations were determined by using the Bradford assay. Proteins were frozen in liquid nitrogen and stored at -80℃.

Site-Directed Mutagenesis of *ytkR2*. The mutagenesis in *ytkR2* was introduced by an overlapping PCR method. Acquired mutants were Y31A, K33A, R47A, R151A, R198A, K202A and R225A. Take Y31A for example. For the first

step, two fragments of *ytkR2* were amplified separately by PCR using the plasmid pTG1313 as template. A 113 bp DNA encoding the N-terminal fragment of *ytkR2* (fragment 1) was amplified using primers ytkR2-F and ytkR2-31NR. Similarly, a 683 bp DNA encoding the C-terminal fragment of *ytkR2* (fragment 2) was amplified using primers ytkR2-31CF and ytkR2-R. For the second step, the whole modified *ytkR2* was amplified using fragments 1 and 2 as templates with primers ytkR2-F and ytkR2-R. Gel-purified PCR products were digested with *Nde*I and *Xho*I and subsequently ligated into *Nde*I/*Xho*I-digested pET37b.

DNA Alkylation by YTM. The plasmid pSP72 was digested with *Eco*RI and then treated in a 50 μL buffer containing 100 mM Tris-HCl (pH=7.2), 5 μL DMSO, 60 μM of YTM and 30 nM of pSP72 at room temperature for 36 h. And another mixture was treated without yatakemycin as control I. The reaction buffer was then directly used as substrate I for glycosylase enzyme assay. Oligonucleotides SUB (5'-GCTAATTCTTTTTGAATTAGC)[16] was synthesized at Invitrogen Shanghai Center and treated in a 50 μL buffer containing 100 mM Tris-HCl (pH=7.2), 5 μL DMSO, 400 μM of yatakemycin and 200 μM of oligonucleotides at room temperature for 36 h. And this reaction buffer was subsequently used as substrate II for enzyme assay.

***In vitro* Glycosylase Activity Assay.** YtkR2 and other enzymes were first treated with 0.1 mM EDTA (pH=8.0) on ice for an hour. A typical reaction buffer contained 1 μM of each protein, 2 μL of substrate I or control I, 50 mM Tris-HCl (pH=7.5), 50 mM NaCl and 10% glycerol and was incubated at 30 ℃ for 10 min. The reaction was stopped at 70 ℃ for 15 min and then centrifuged at 12, 000 rpm for 5 min. The supernatant was applied to 1% agarose gel. Another reaction buffer contained 5 μL of Substrate II and 10 μM of YtkR2 or other proteins and treated in the same way as above. The supernatant of reaction was applied to HPLC and LC-MS for further analysis.

***In vivo* Assay for YTM Sensitivity.** BL21(DE3) carrying plasmid pET37b or pTG1313 was inoculated into 5 mL of LB and cultured until OD_{600}=0.6. Same volume of the cultured cells were then plated onto LB plates supplemented with 50 ng/mL kanamycin and different concentrations of YTM or MMS.

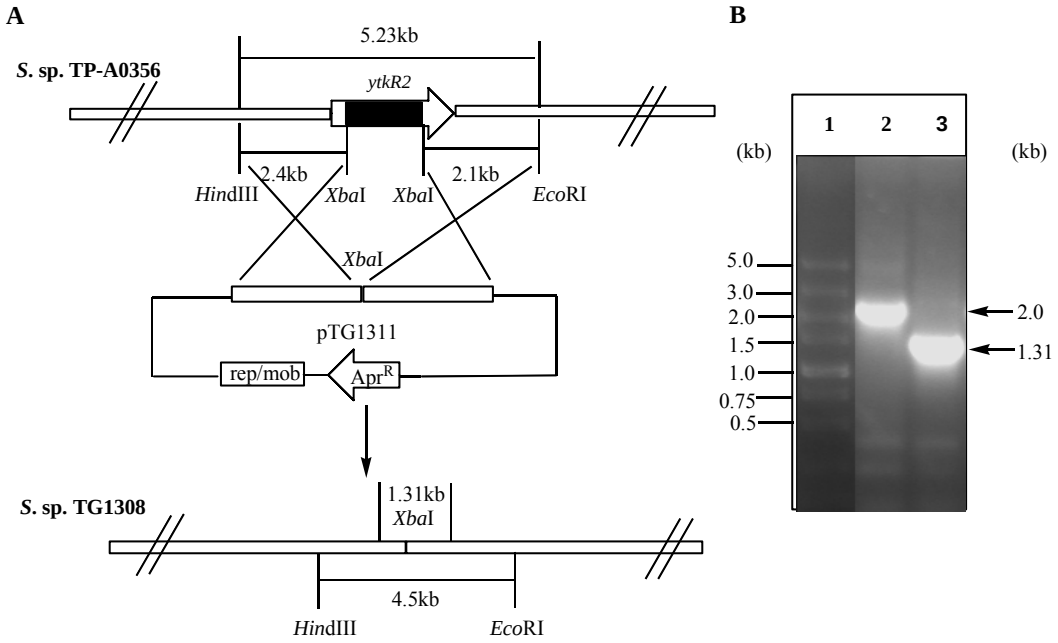
Surface Plasmon resonance (SPR) analysis. SPR analysis was carried out using the ProteOn XPR36 Protein Interaction Array System (Bio-Rad Laboratories, Hercules, CA, USA), an SPR technology-based imaging optical biosensor. The protein YtkR2 was immobilized on the GLH surface in different channels. The oligo DNA (5'-GCTAAT-TCTTTTTGAATTAGC) was diluted with PBS (50 mM, pH 7.5) to different concentrations (0, 4.6 μM, 10 μM, 20 μM, 28 μM) and pass across the YtkR2-immobilized surface. The binding measurements were performed with PBS as the continuous running buffer at room temperature. Relative binding responses were determined by measuring changes in refractive index levels before and after the addition of oligo DNA.

Molecular Modeling. Homology modeling of the YtkR2 structure was carried out by using the I-TASSER on-line server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/). The complete sequence of YtkR2 was submitted to this server as inputs. Then, the structural model was generated through a multi-step process, including template selection, reassembly of threading aligned regions, loop building, and further optimizations. The top-ranked YtkR2 model was selected for further uses in our study. To construct the structural model of the YTM alkylated DNA-YtkR2 complex, the AlkD-DNA complex structure (PDB entry 3JX7) was used as the template. Here, the 3-deaza-3-methyladenine in the

DNA template was altered to standard adenine (A7). Then, the YtkR2 structure generated by I-TASSER was superimposed onto the AlkD structure in this modified AlkD-DNA complex structure. Side chains of several residues on YtkR2 near the binding site was adjusted manually to resemble the conformations of their counterparts on AlkD. The DNA duplex was manually docked into the binding site on YtkR2. The YTM molecule was covalently connected to the N3 nitrogen on adenine to obtain a YTM-alkylated adenine residue (**3**). The adenine moiety on **3** molecule was then superimposed onto the A7 adenine on the DNA duplex. Then, the resulting YtkR2-YTM-DNA complex structure was subjected to energy minization to remove possible steric clashes on the binding interface. All molecular modeling operations described above were executed by using the SYBYL software (version 7.3). Energy computations were all executed by using the AMBER FF99 force field implemented in SYBYL.

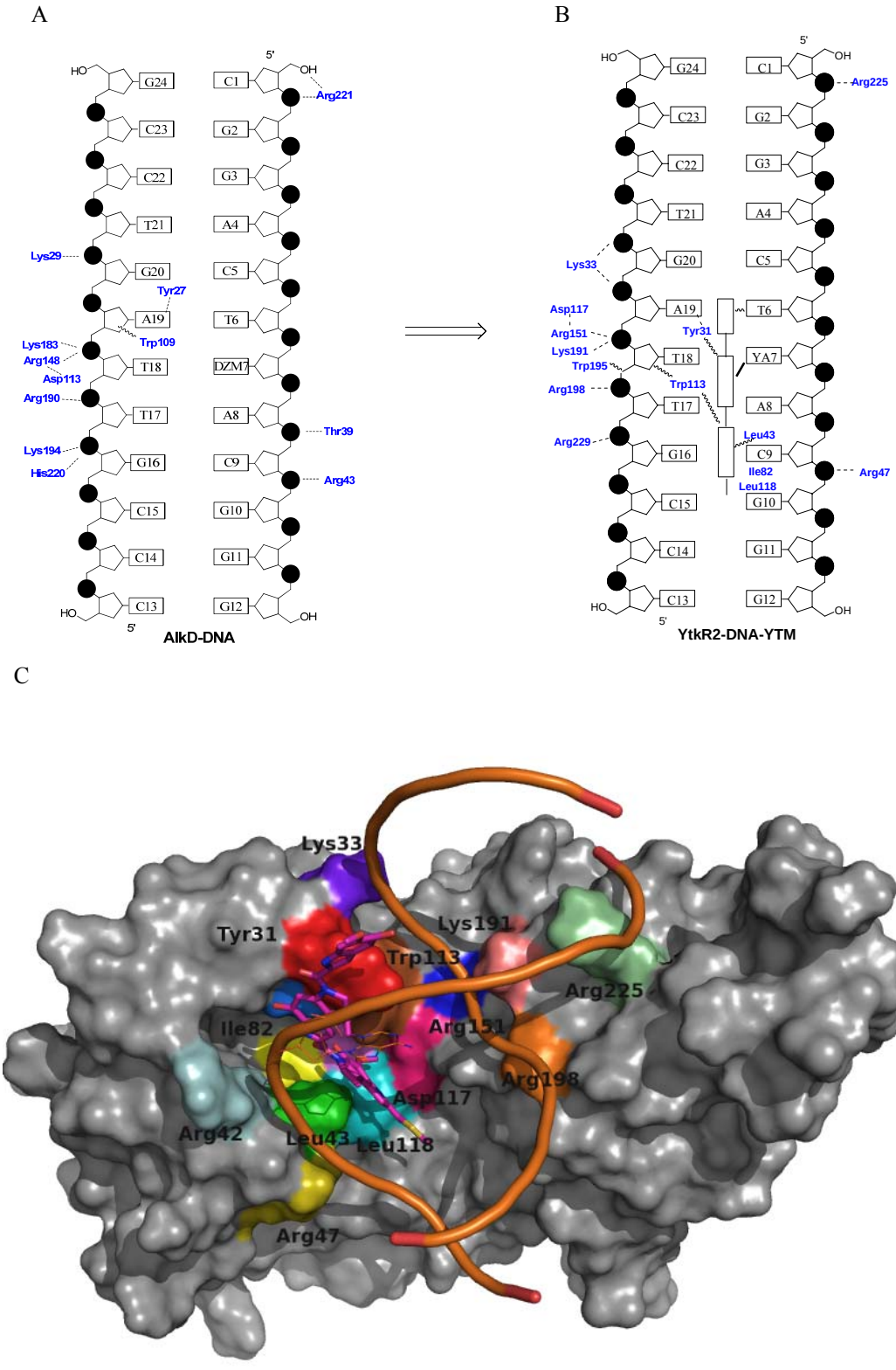
As further refinement, this YtkR2-YTM-DNA complex structure was subjected to molecular dynamics simulation in explicit water. The complex structure was solvated in TIP3P water box, and the entire system was optimized by 500 steps of conjugated-gradient energy minimization. This system was then subjected to a 6 ns-long molecule dynamic simulation carried out by using the Desmond module in the Schrodinger software (version 2010). To keep the overall shape of the DNA duplex, a position restraint on the four terminal residues on the DNA duplex with a force constant of 50 kcal/mol·$Å^2$ was applied in simulation. Final model of the YtkR2-YTM-DNA complex was evaluated by Procheck, QMEAN, Profile 3D and DOPE. All these tools gave acceptable scores for this complex structural model.

Figure S1.



Construction of *ytkR2* gene replacement mutant strain *Streptomyces* sp TG1308 *via* homologous recombination. (A) A 705 bp gene fragment in *ytkR2* gene was deleted by in-frame double crossover. (B) Genomic DNA from wild type and TG1308 mutant strain was used as template, respectively. PCR analysis of the genotype using 5'-GAGAAGGCCACCATCTTC and 5'-GGACATCCTCGGTATCGC as primers. Lane 1, DNA marker; lane 2, wild type; lane 3, *Streptomyces* mutant strain TG1308.

Figure S2.



The interactions between: (A) AlkD and DNA (B) YtkR2 and DNA-YTM (YTM showed in box-connected model). The dash lines and wavy lines stand for the hydrogen bond and hydrophobic interaction between the residues in protein and the residues in DNA, respectively; (C) The model showing the interaction between residues of YtkR2 and DNA-YTM. The key residues in YtkR2 were rendered by different colors on the YtkR2 surface. The figure S2 (C) in SI and figure 3(C-E) in the main text were generated by Pymol.