Method and computation

Molecular docking calculation

The crystal structure of SrtB was obtained from the Protein Data Bank (PDB), and PDB code 1NG5 was used as the initial coordinates for the molecular docking calculations using AutoDock 4.0 [1, 2]. The Lamarckian genetic algorithm (LGA) was applied for the docking calculations. All of the torsional bonds of the drug were free to rotate, while SrtB was held rigid. The polar hydrogen atoms were added for SrtB using the AutoDock tools, and the Kollman united atom partial charges [2] were assigned. A total of 150 independent runs were carried out with a maximum of 25,000,000 energy evaluations and a population size of 300. A grid box of dimensions (40×40×40) with a spacing of 1 Å was created and centred on the mass centre of the ligand. Energy grid maps for all possible ligand atom types were generated by using Autogrid 4 before performing docking.

Molecular dynamics simulation

All of the simulations and analyses of the trajectories were performed with Gromacs 4.5.1 software [3] using the Amber99sb force field and TIP3P water model [4]. The SrtB-coptisine system was initially energy-relaxed with 2000 steps of the steepest-descent energy minimization and 2000 subsequent steps of conjugate-gradient energy minimization. The system was then equilibrated by a 500 ps molecular dynamic run with positional restraints on both the protein and ligand to allow relaxation of the solvent molecules. The first equilibration run was followed by a 100 ns MD run without position restraints on the solute. The first 20 ns of the trajectory were not used in the subsequent analysis for minimization of convergence artefacts. Equilibration of the trajectory was checked by monitoring the equilibration of quantities, such as the root-mean-square deviation (rmsd) with respect to the initial structure, internal protein energy and fluctuations calculated for different time intervals. The electrostatic term was described with the particle mesh Ewald algorithm. The LINCS [5] algorithm was used to constrain all of the bond lengths. For the water molecules, the SETTLE algorithm [5] was used. The dielectric permittivity ε was set as 1, and a time step of 2 fs was used. All atoms were given an initial velocity as

determine from the Maxwell distribution at the desired initial temperature of 300 K. The density of the system was adjusted during the first equilibration runs under NPT conditions by weak coupling to a bath of constant pressure (P_0 = 1 bar, coupling time τ_P = 0.5 ps) [6]. In all of the simulations the temperature was maintained close to the intended values by weak coupling to an external temperature bath with a coupling constant of 0.1 ps. The proteins and rest of the system were coupled separately to the temperature bath. The structural cluster analysis was carried out using the method described by Daura and co-workers with a cutoff of 0.25 nm [6].

The parameters of coptisine was estimated with the antechamber program [7] and the RESP partial atomic charges from the Amber suite [8]. Analysis of the trajectories was performed by using the VMD, PyMOL and Gromacs analysis tools.

Calculation of the binding free energy

In this work, the binding free energies were calculated using the MM-PBSA approach [9, 10] supplied with the Amber 10 package. We chose a total number of 100 snapshots evenly from the last 70 ns on the MD trajectory with an interval of 10 ps. The MM-PBSA method can be conceptually summarized as one

$$\Delta G_{bind} = \Delta G_{complex} - [\Delta G_{protein} + \Delta G_{lig}] \quad (1)$$

$$\Delta G_{bind} = \Delta H - T\Delta S \quad (2)$$

which the ΔH of the system is composed of the enthalpy changes in the gas phase upon complex formation (ΔE_{MM}) and the solvated free energy contribution (ΔG_{sol}), while $-T\Delta S$ refers to the entropy contribution to the binding. Eq. 2 can then be approximated as shown in Eq. 3:

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{sol} - T\Delta S \quad (3)$$

where ΔE_{MM} is the sum of the van der Waals (ΔE_{vdw}) and electrostatic (ΔE_{ele}) interaction energies.

$$\Delta E_{MM} = \Delta E_{vdw} + \Delta E_{ele} \quad (4)$$

In addition, ΔG_{sol}, which denotes the solvation free energy, can be computed as the sum of an electrostatic component ($\Delta G_{ele,sol}$) and a nonpolar component ($\Delta G_{nonpolar,sol}$), as shown in Eq. 5:

$$\Delta G_{sol}=\Delta G_{ele,sol}+\Delta G_{nonpolar,sol} \quad 5$$

The interactions between coptisine and each residue in the binding site of SrtB with coptisine were analysed using the MM-PBSA decomposition process applied in the MM-PBSA module in Amber 10. The binding interaction of each ligand-residue pair includes three terms, namely, the Van der Waals contribution (ΔE_{vdw}), electrostatic contribution (ΔE_{ele}) and solvation contribution (ΔE_{sol}). All of the energy components were calculated using the same snapshots as the free energy calculation.

Fluorescence quenching analyze the binding affinity of coptisine with $SrtB_{\Delta 30}$-WT and its mutants

Fluorescence quenching method was used to measure the binding constants (K_A) of coptisine with the binding site of $SrtB_{\Delta 30}$-WT, $SrtB_{\Delta 30}$-R115A, $SrtB_{\Delta 30}$-N116A and $SrtB_{\Delta 30}$-I182A. The emission wavelength was 326nm with a 5nm slit width and the excitation wavelength was 280nm with a 5nm slit width. The details of the measurement procedure referenced the method previously described [11, 12].

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