SUPPORTING INFORMATION

The Intrinsic Conformational Plasticity of Native EmrE Provides a Pathway for Multidrug Resistance

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SI FIGURE 1

(A) Primary sequence of EmrE. (B) W31, W45, and W76 residues highlighted on the X-ray crystal structure of TPP⁺ bound EmrE₁ reconstructed from Cα coordinates using REMO.² As observed in Figure 3A, these residues were sensitive to the temperature dependent conformational motion of native EmrE.
SI FIGURE 2

$^1$H/$^{15}$N TROSY-HSQC NMR spectra of EmrE at a range of indicated temperatures at 800 and 900 MHz (red: native state; black: TPP$^+$). Acquisition parameters for the data at 800 MHz: spectral widths (acquisition or evolution time) of 11161 Hz (45.9 ms) and 2757 Hz (18.1 ms) for $^1$H and $^{15}$N with a 1 s recycle delay time. Data at 900 MHz: spectral widths (acquisition or evolution time) of 12626 Hz (50 ms) and 3102 Hz (16.1 ms) for $^1$H and $^{15}$N dimensions with a 1 s recycle delay time.
SI FIGURE 3

Temperature dependent chemical shift changes of native EmrE in DLPC/DHPC bicelle. $^{1}$H/$^{15}$N-TROSY spectra of [U-$^{15}$N] EmrE (protonated) in DLPC/DHPC bicelle show peak splitting for indole peaks at 25 °C that was similar to that observed in DMPC/DHPC bicelles.
$^{1}$H/$^{13}$C HMQC NMR spectra of EmrE at a range of indicated temperatures at 800 and 900 MHz (red: native state; black: TPP$^+$). Acquisition parameters for the data at 800 MHz: spectral widths (acquisition or evolution time) of 10417 Hz (98.3 ms) and 5232 Hz (19.1 ms) for the $^1$H and $^{13}$C dimensions with a 2 s recycle delay time. Data at 900 MHz: spectral widths (acquisition or evolution time) of 11719 Hz (87.4 ms) and 5889 Hz (17.0 ms) for the $^1$H and $^{13}$C dimensions with a 2 s recycle delay time.
SI FIGURE 5

Methyl cross peaks in $^1$H/$^{13}$C HMQC NMR spectra with temperature variation. The peaks are in fast exchange at the highest temperature (45 °C) then progressively shifts to the slow/slow-intermediate timescale at the lowest temperature (25 °C) (red: native EmrE; black: TPP$^+$ bound EmrE). Only the TPP$^+$ data is shown at 45 °C for clarity of the effect on the native EmrE spectra at lower temperatures.
SI FIGURE 6

Plots of experimental (red) and fitted (blue) 1D spectra for native EmrE that are reported in SI Table I.
SI FIGURE 7

1D PUREX difference spectra for native and TPP$^+$ bound EmrE in oriented DMPC/DHPC lipid bicelles (q=3.2). The ZZ-mixing times in msec are listed on the top of the figure. All experiments were acquired with a fixed tau modulation time of 250 µsec as described previously. The integrated intensities from 40-250 ppm of these spectra and others are shown in Figure 4 in the main text.
SI FIGURE 8

TM1 tilt angle fitting using the PDB 2I68 backbone model of EmrE. The score was obtained by rotating the TM1 helices every 2° with respect to the magnetic field (z-axis of pdb) and calculating the deviation between the experimental and calculated chemical shifts and dipolar couplings:

$$\text{Score}_i = \sum_i \sqrt{(CS_{\text{calc},i} - CS_{\text{obs},i})^2 + [10(DC_{\text{calc},i} - DC_{\text{obs},i})]^2}$$

CS is the $^{15}$N anisotropic chemical shift, DC is the $^1$H-$^{15}$N dipolar coupling, and i is the residue (i.e., V15, T18, T19, and M21). A lower score reflects better agreement with the structure. The score was calculated for residues V15, T18 and T19 for monomer A, while monomer B utilized V15, T18, T19, and Met21. The following tilt angles were found to be optimal for monomers A and B, respectively: 33 ± 4° and 16 ± 4°. Note that the A and B nomenclature corresponds to chain A or B of 2I68.
SI FIGURE 9

Fitted mixing curve plots from MAS data of native EmrE acquired at 9 °C in DMPC lipid bilayers for Leu83 and Leu104. The data were obtained with $^{13}$C/$^{13}$C PDSD$^5$ exchange experiments on $[^{13}$C$_\alpha$,$^{15}$N-Leu] native EmrE. The four cross-peaks are shown in Figure 6. The $k_{ex}$ values were obtained in a global manner by fitting all four curves simultaneously.
SI FIGURE 10

(A) The crosslinking reaction with the BMPS heterobifunctional linker was ~83% on native EmrE as calculated from the SDS-PAGE gel. (B) Trp fluorescence binding experiments of crosslinked EmrE dimer (CL-EmrE) to TPP$^+$ at 25 °C in DDM micelles. The concentration of CL-EmrE dimer was 1.8 µM and the fit $K_d$ was 187 nM. The excitation wavelength was 270 nm and the emission was followed at 330 nm using a Molecular Devices SpectraMax with a 96-well plate.
SI FIGURE 11

Oriented solid-state NMR PUREX data in aligned bicelles as supplementary information for Figure 4. Two trials were carried out with separately prepared EmrE samples with the \( k_{ex} \) value fitted in a global fashion between the two datasets. Panels A and C correspond to native EmrE, while B and D are of the TPP\(^+\) bound form. Note the different x-axis values between the native state and TPP\(^+\) bound panels. Each of the two trials resulted in essentially the same scaling factors between the free and bound forms (i.e., panels A and B level off at \(~3\); panels C and D are \(~1.8\)), which indicates that the same number of residues are involved in the conformational change for the substrate-free and TPP\(^+\) bound forms of EmrE. The fitted \( k_{ex} \) value is reported in SI Table I.
SI FIGURE 12

Differential scanning calorimetry thermogram data obtained with DMPC in the presence and absence of TPP⁺. The TPP⁺:lipid molar ratio was the same as that used for the EmrE samples. Fitted values are shown in SI Table II.
SI FIGURE 13

Trp indole region of $^1$H/$^{15}$N TROSY-HSQC spectra acquired for WT-EmrE and CL-EmrE in the absence and presence of TPP$^+$. All data were acquired at 600 MHz $^1$H frequency. The CL-EmrE spectra are also shown in Figure 7 in the main text.
SI FIGURE 14

Trp indole region of $^1$H/$^{15}$N TROSY-HSQC spectra acquired at 600, 800, and 900 MHz at 45 °C. The differential behavior of the peak intensities is characteristic of the field dependence to the chemical shift difference ($\Delta \omega$). The broadening in the 900 MHz spectrum is indicative of intermediate chemical exchange, while the 600 MHz experiment resembles a fast-intermediate regime. The black peaks are for native EmrE, while the grey outlined peaks in the background correspond to the TPP$^+$ bound protein.
SI TABLE I

| Temperature (°C) | $k_{ex}$ (s$^{-1}$) | Method                      |
|-----------------|---------------------|-----------------------------|
| 45              | 500 ± 200           | Solution NMR               |
| 40              | 270 ± 30            | Solution NMR               |
| 35              | 150 ± 9             | Solution NMR               |
| 30              | 97 ± 4              | Solution NMR               |
| 25              | 40 ± 3              | Solution NMR               |
| 37              | 350 ± 60            | O-SSNMR (aligned bicelles) |
| 9               | 1.9 ± 0.5           | MAS (bilayers)             |

Fitted conformational exchange rates for native EmrE. The solution NMR-derived exchange rates were obtained from global fitting in isotropic bicelles. The global fits at 35 °C, 40 °C, and 45 °C were carried out with seven sites (note: indole W76, methyl I5 and methyl I37 spectra were excluded due to fast exchange at these temperatures), while all 10 lineshapes were included for the global fit at 25 °C and 30 °C.

The molar ratio of populations A and B were set to 0.5 and $\omega_A$ and $\omega_B$ were set as $\Delta \omega_{AB} (= \omega_A - \omega_B)$, which was the same for all temperature values. This value was extracted from the distance between peak positions in native EmrE at the lowest temperature that was closest to the slow exchange limit (20 °C). 0.03 sec. was set for upper limit for $T_{2A}$ and $T_{2B}$. 
Fitted parameters from the DSC data carried out on wild-type and crosslinked EmrE (CL-EmrE). 

\( T_m \), \( \Delta H_m \), and \( \Delta T_{1/2} \) correspond to the temperature, enthalpy, and half height of the main phase transition, respectively.

| Sample            | \( T_m \) (°C) | \( \Delta H_m \) (kcal/mol) | \( \Delta T_{1/2} \) (°C) |
|------------------|----------------|-----------------------------|---------------------------|
| WT-native        | 22.96 ± 0.05   | 2.7 ± 0.1                   | 4.0 ± 0.1                 |
| WT-TPP\(^+\)     | 22.96 ± 0.05   | 2.8 ± 0.1                   | 2.0 ± 0.1                 |
| CL- no TPP\(^+\) | 22.74 ± 0.05   | 2.3 ± 0.1                   | 3.4 ± 0.1                 |
| CL-TPP\(^+\)     | 23.25 ± 0.05   | 2.4 ± 0.1                   | 1.8 ± 0.1                 |
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