Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information

TITLE: DNA Condensation in Live *E. Coli* Provides Evidence for Transertion

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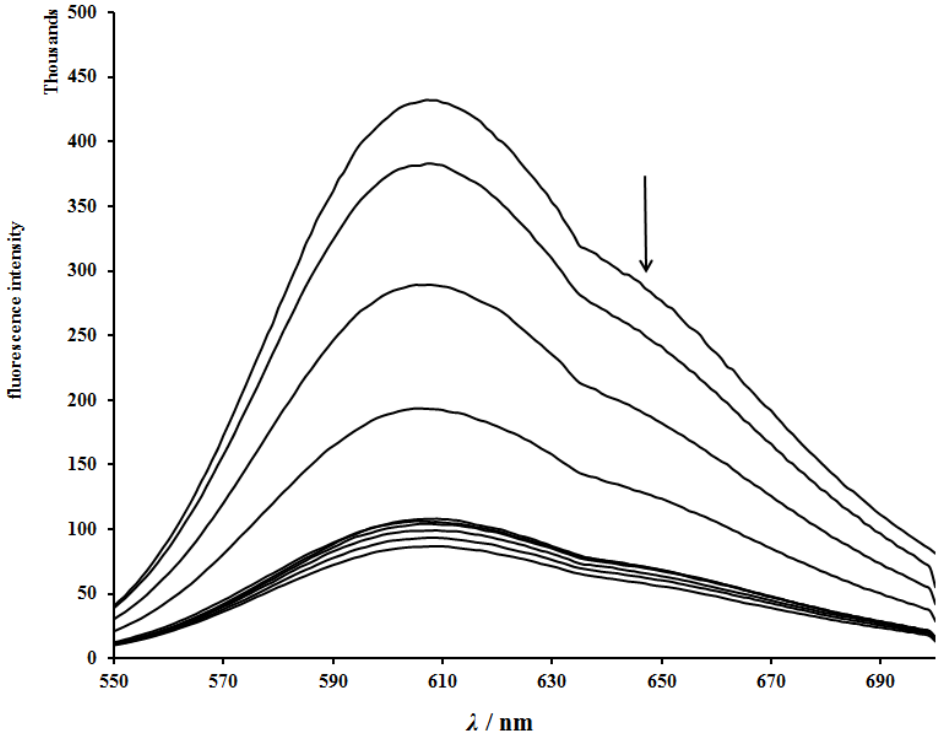

Figure S1. Changes in the emission spectra of $Rubb_{12}$-tetra with successive additions of CT-DNA.

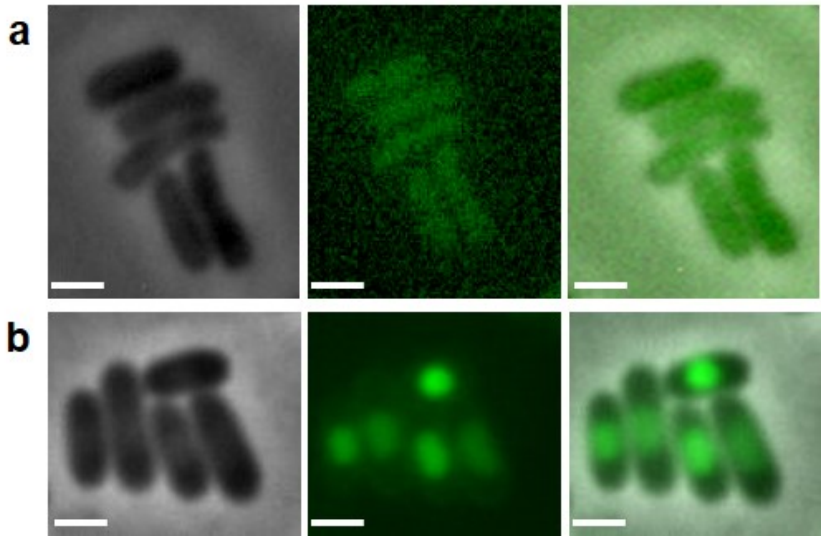

Figure S2. Effect of anti-bacterial agents on the nucleoid of the *E. coli* cells in the absence of $Rubb_{12}$-tetra (control experiments). The fluorescence microscopy images are: (a) top panel – control experiment where the cells are treated with Rifampicin (RIF) for 30 minutes and then stained with DAPI; (b) bottom panel – control experiment where the cells are treated with Chloramphenicol (CAM) for 30 minutes and then stained with DAPI. Images from left to right; phase contrast; fluorecence – DAPI (coloured green); and merged (DAPI + phase contrast). Scale bar = 5 μm.

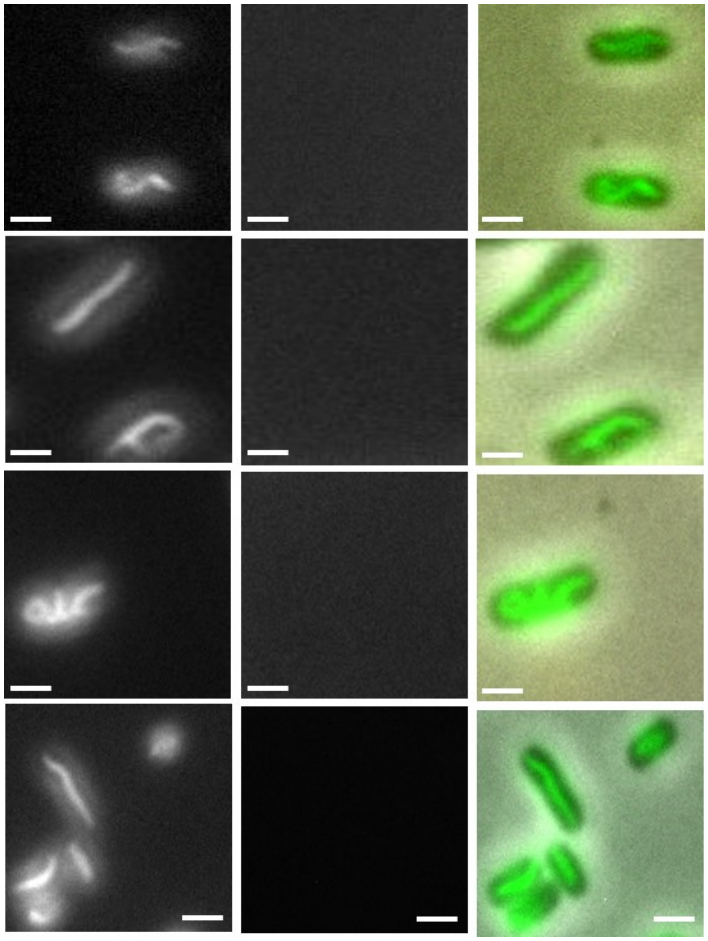

Figure S3. Fluorescence microscopic images of rifampicin treated *E. coli* cells incubated with $Rubb_{12}$-tetra for 15 minutess and then stained with DAPI. Images from left to right: fluroscence – DAPI (coloured white); phosphorescence – $Rubb_{12}$-tetra; and merged (DAPI {coloured green} + phosphorescence). Scale bar = 5 μm.

Experimental methods

Materials

$Rubb_{12}$-tetra was synthesised as described previously.[1] Luria Broth base was purchased from BD Difco. DNA selective stain DAPI (4′,6-diamidino-2-phenylindole) was obtained from Molecular Probes, Invitrogen. Rifampicin and chloramphenicol were purchased from Sigma-Aldrich.

Bacterial strains and growth conditions

As the *E. coli* strain MG1665 was used in a previous study of the intracellular localisation of $Rubb_{16}$,[2] the present study was also carried out using this bacterium. The bacterial strain was grown on Luria Broth (LB) agar plates at 37 °C. A bacterial culture was obtained by inoculating bacteria in LB media and incubating overnight in a shaking incubator at 37 °C. The overnight culture was then diluted to a suspension with an optical density at λ600 nm (OD_{600}) of approximately 0.05. A bacterial log phase culture was obtained by continuing the incubation of this suspension for approximately 2 h until the OD_{600} reached 0.5.

MIC assay

The minimum inhibitory concentration (MIC) of $Rubb_{12}$-tetra against *E. coli* MG1665 was determined by the broth microdilution method as outlined in the CLSI guidelines.[3] The overnight bacterial culture was diluted to a concentration of $4\sim8 \times 10^5$ cfu/mL. $Rubb_{12}$-tetra was serially diluted in LB media on a sterile 96-well plate with a final volume of 100 μL. The bacterial suspension (100 μL) was added to each well and the final concentration range of $Rubb_{12}$-tetra was from 0.125 to 128 μg/mL. The plate was then placed in a static incubator at 37 °C for 14-16 h before the MIC results were read.

Drug treatment and staining protocols

A $Rubb_{12}$-tetra stock solution was prepared in Milli-Q water at a concentration of 128 mg/L. $Rubb_{12}$-tetra was added to the log phase bacterial culture and incubated for either 0.5 h or 1 h. The concentrations of $Rubb_{12}$-tetra used in this study ranged from 0.5×MIC to 2×MIC. After incubation with $Rubb_{12}$-tetra, the cells were washed twice with a phosphate buffer solution before further treatment or preparation for slides. For the co-localisation experiment with DAPI, *E. coli* cells treated with $Rubb_{12}$-tetra were incubated with DAPI at room temperature for 15-30 min before being loaded onto agarose pads on slides for microscopy. The concentrations of DAPI used in the co-localisation assays were 20 mg/L.

Rifampicin and chloramphenicol were initially dissolved in 20 μL of ethanol and then diluted in Milli-Q water to a concentration of 2 mg/mL and 20 mg/mL, respectively. Bacterial cells in the log phase of growth at 37 °C were treated with either of the inhibitors at a concentration of 128 mg/mL for 30 min and then with $Rubb_{12}$-tetra for 15 min.

Live cell microscopy

All live cell microscopy was performed by placing cells on 2% (w/v) agarose pads (prepared with identical media to that in which the cells were grown) within a 65 μL Gene Frame (Thermo Fisher Scientific). Luminescent images were obtained using a Zeiss Axioplan 2 fluorescence microscope (Carl Zeiss) as described previously.[4,5] DAPI fluorescence and $Rubb_{12}$-tetra phosphorescence were visualised with filter sets 02 and 488015 (Carl Zeiss), respectively. Images were analysed and processed using AxioVision version 4.8 (Carl Zeiss).

$Rubb_{12}$-tetra phosphorescence quenching by calf-thymus DNA (CT-DNA)

The effect of CT-DNA on the $Rubb_{12}$-tetra phosphorescence was determined by monitoring the $Rubb_{12}$-tetra emission upon successive additions of CT-DNA (r = 0.5 to 4.5) to an aqueous solution of $Rubb_{12}$-tetra (2 μM; volume 3000 μL).

References

1. A. K. Gorle, M. Feterl, J. M. Warner, L. Wallace, F. R. Keene and J. G. Collins, *Dalton Trans.*, 2014, **43**, 16713.
2. F. Li, E. J. Harry, A. L. Bottomley, M. D. Edstein, G. W. Birrell, C. E. Woodward, F. R. Keene and J. G. Collins, *Chem. Sci.*, 2014, **5**, 685.
3. P. A. Wayne and Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement M100–S19*, C.L.S.I., USA, 2009.
4. P. C. Peters, M. D. Migocki, C. Thoni and E. J. Harry, *Mol.Microbiol.*, 2007, **64**, 487.
5. S. Moriya, R. A. Rashid, C. D. Andrade Rodrigues and E. J. Harry, *Mol. Microbiol.*, 2010, **76**, 634.