Interplays between copper and *Mycobacterium tuberculosis* GroEL1

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Supplementary figure legends

**Figure S1.** The impact of Zn$^{2+}$ (A) and Cd$^{2+}$ (B) on the various *M. bovis* BCG strain biofilm formation. Wild type (wt) *M. bovis* BCG, Δ*groEL1* (KO) BCG, KO complemented (compl.) BCG strains grown for 26 days in 3.5% glycerol Sauton’s medium in the absence or presence of various concentration of ZnCl$_2$ or CdCl$_2$. The figure is representative of three independent experiments.
Figure S2. Recombinant protein purity and integrity determination.

(A). Protein purity determination by SDS-PAGE. a-c: Protein fractions from size exclusion chromatography, (a) GroEL1; (b) GroEL1ΔHis; (c) GroEL2. (d) Final analysis of protein purification. Lane 1: GroEL1; lane 2: GroEL1ΔHis; lane 3: GroES. Lane M: molecular mass standards; FX: fraction number. The symbol “#” indicates the intact GroEL1, and “Δ” indicates the GroEL1 N-terminus degradation. The figure is the representative from at least three independent experiments. (B). Denaturing ESI mass spectrum of purified proteins. (a) GroEL1; (b) GroEL1ΔHis; (c) GroEL2; (d) GroES. The inserts represent the mass spectra obtained after deconvolution of the raw data using the MaxEnt1 software. The experimental values agree with the theoretical molecular masses.
Figure S3. Native nano-ESI mass spectra of GroEL1 in 10 mM ammonium acetate, pH 6.9. The protein concentration was 10 µM. [M + 18H]$^{18+}$ charge state of (A) the apo form, and in the presence of one molar equivalent of (B) Zn$^{2+}$, (C) Ni$^{2+}$, and (D) Co$^{2+}$. The vertical dotted line and the asterisk indicate the position of the apo and holo form with one metal atom bound, respectively.
Figure S4. Native nano-ESI mass spectra of GroEL1 (7.5 µM) in 500 mM ammonium acetate in the absence or presence of Cd$^{2+}$. The cadmium bound GroEL1 peak is indicated with an asterisk.
Figure S5. GroEL1 oligomeric state determination by native mass spectrometry. The spectra were recorded at a final protein concentration of 7.5 µM in the absence or presence of Cu²⁺ (7.5 or 30 µM).
Figure S6. Protection of GroEL1 from limited trypsin digestion in a Cu$^{2+}$ concentration dependent manner. The reaction mixture contained 5 µg GroEL1, 0.0015 µg trypsin, in the presence or absence of various concentrations of CuCl$_2$. The reaction was stopped at different times (0, 1, 3, 10, 20, 30 min) by adding PMSF. The solutions were analysed by 15% SDS-PAGE. Lane 1 and 16: molecular mass standards; lane 2, 15: protein alone. +: molar ratio of Cu$^{2+}$ to protein (2:1); ++: molar ratio of Cu$^{2+}$ to protein (3:1). The figure is the representative of three independent experiments.
Figure S7. *E. coli* GroEL ATPase activity in the presence of Cu$^{2+}$ and Co$^{2+}$.

The reactions (50 μL), containing 2.7 μg GroEL, 10 mM KCl, 2 mM ATP, 10 mM MgCl$_2$ in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, in the absence or presence of Cu$^{2+}$ and Co$^{2+}$ were incubated 1 hour at 37 °C. The absorbance was recorded at 700 nm. The mean from three independent experiments for individual data sets was calculated and plotted along with the standard deviation, considering the activity measured in the absence of metal ions as 100%.
### Supplementary Table

Table S1. Plasmids and oligonucleotide primers

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<th>Oligonucleotide</th>
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<th>Plasmids</th>
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<td>pMtGroEL1</td>
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<td>Cpn60.1-R1</td>
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