

Supplemental material

Functional Dissection of Inter-subunit Interactions in the EspR Virulence Regulator of *Mycobacterium tuberculosis*

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Running title: Functional dissection of EspR-EspR interactions

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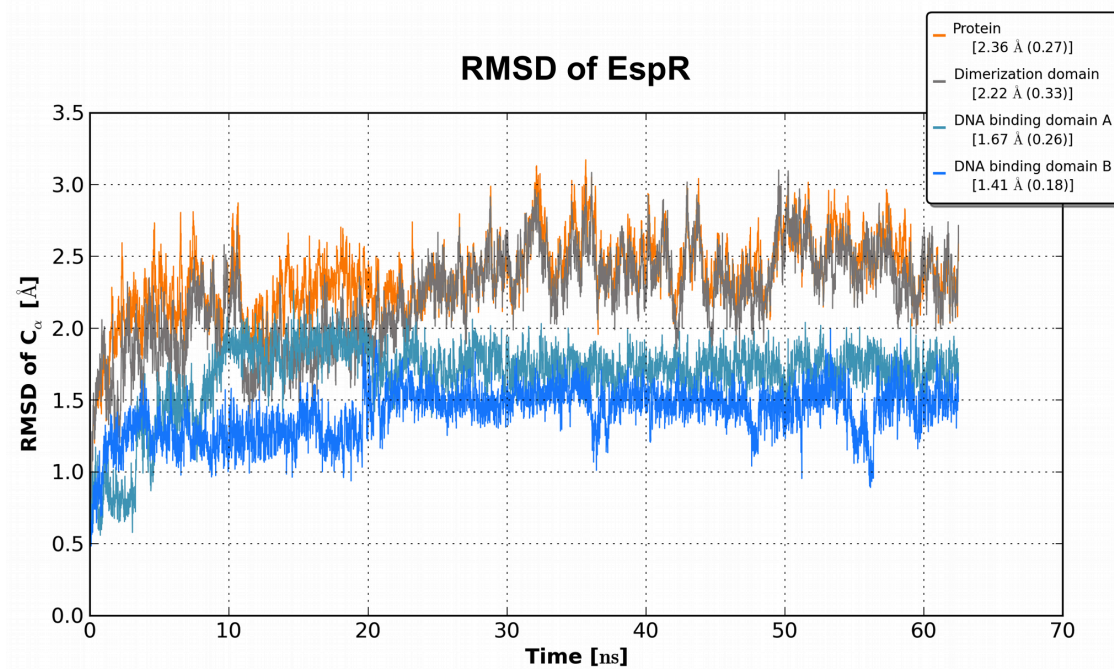
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FIG S1 Root mean square deviation (RMSD) calculated from molecular dynamic simulations of the (A) wt EspR and (B) EspR Δ 10 models. Calculations performed on C $_{\alpha}$ atoms for different portions of the systems (labels in the upper-right box of each graph). The overall RMSD for each portion, as well as the corresponding standard deviation (in brackets) are reported next to each label. The analysis was done with ProDy and plotted with Matplotlib Python libraries.

A



B

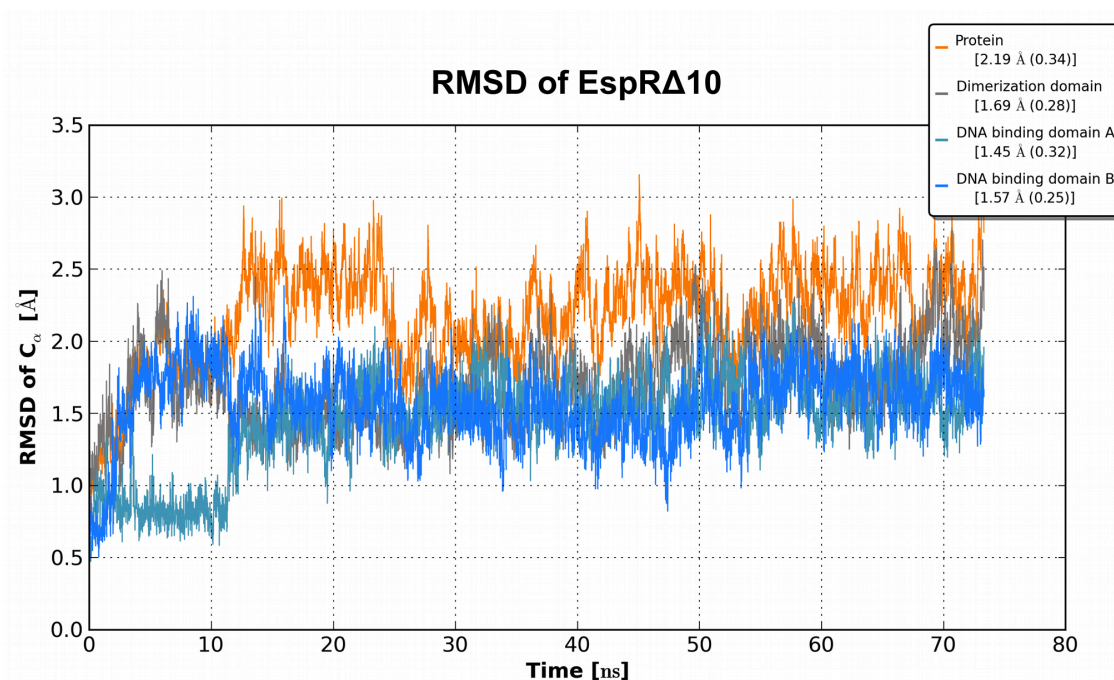
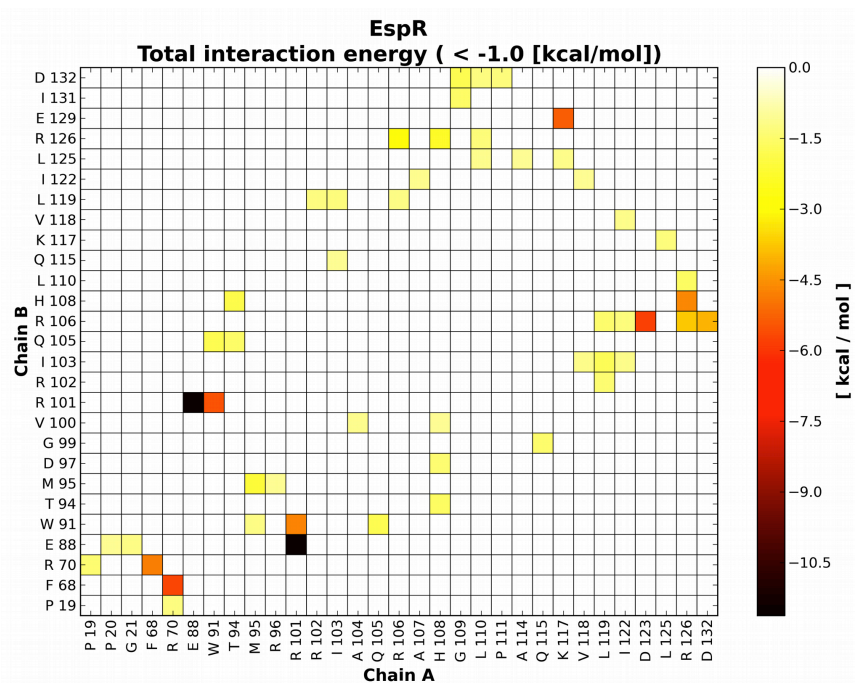


FIG S2 Binding energy decomposition of EspR protomer-protomer interactions. The heat maps report pairwise binding energies calculated using a single-trajectory MM/GBSA binding free energy decomposition on molecular dynamics trajectories of (A) wt EspR and (B) EspR Δ 10 (500 evenly spaced snapshots taken from the last 30 ns of the corresponding equilibrated trajectories). For clarity, only significant values below -1 kcal/mol are reported.

A



B

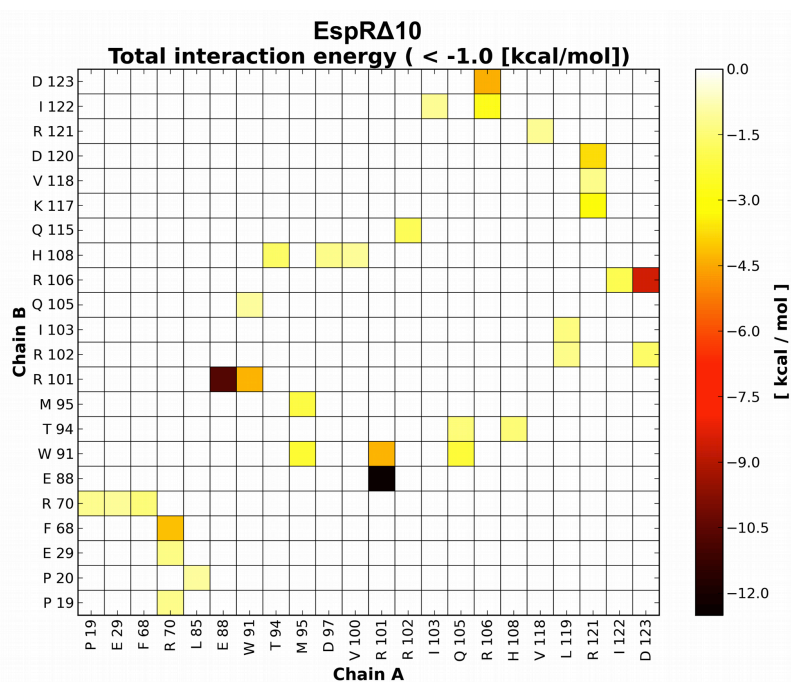


FIG S3 AFM imaging of DNA and EspR proteins alone. In the absence of EspR, the DNA molecules have a "relaxed" appearance and a uniform height (0.85 ± 0.15 nm). Protein particles have a wider height profile, ranging from 0.5 to 1.3 nm, probably due to multiple orientations and varying oligomerization levels.

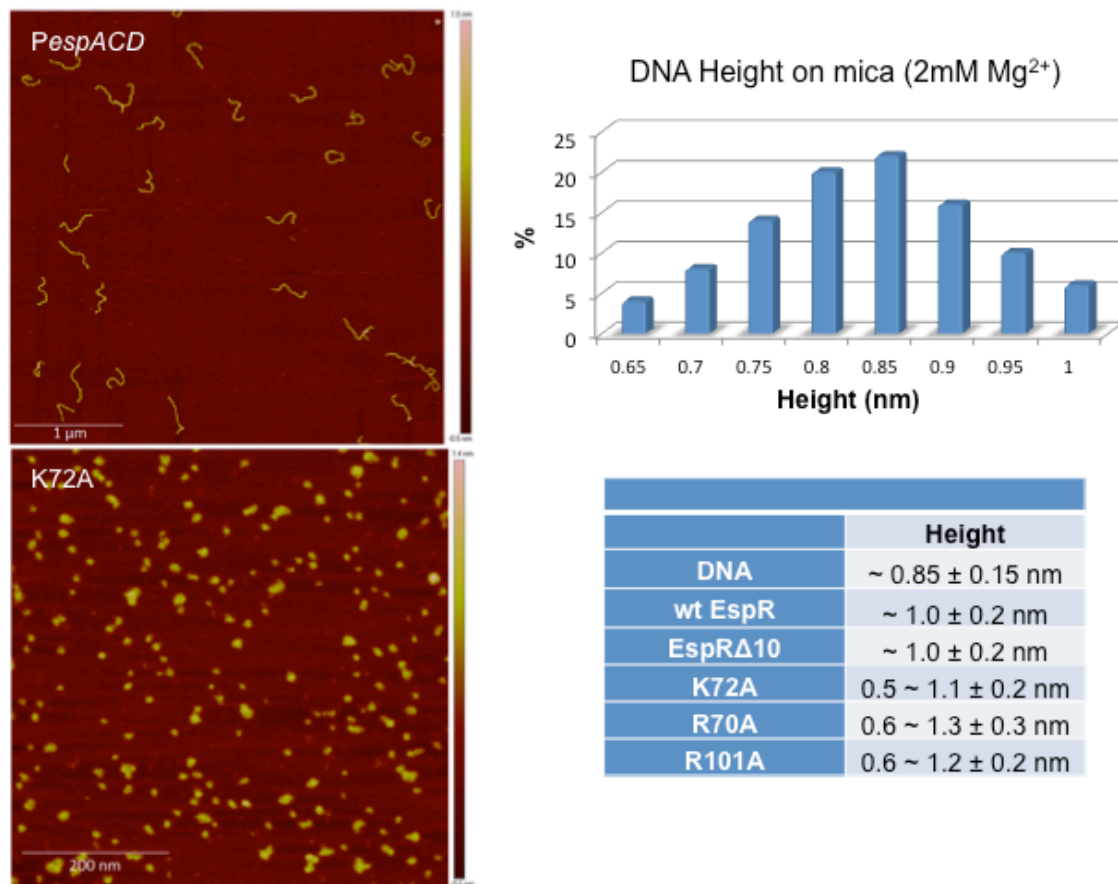
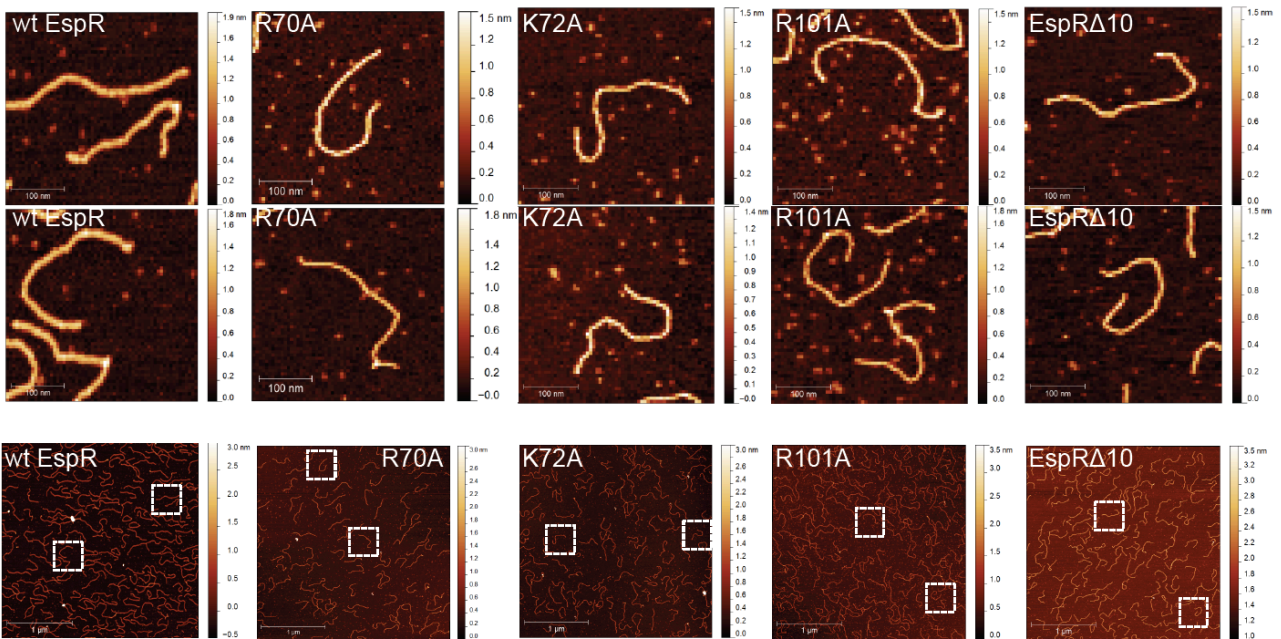
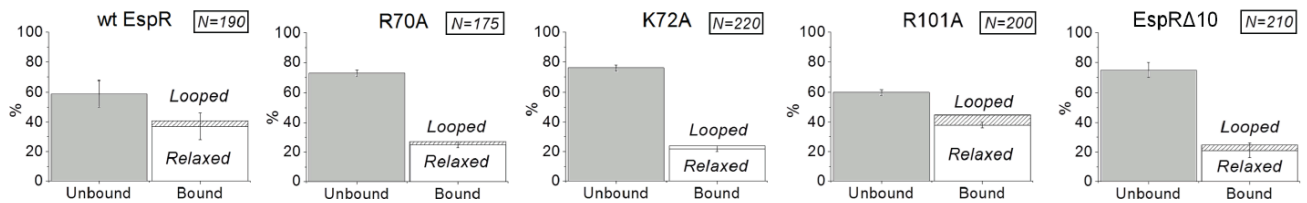


FIG S4 Control AFM images of EspR variants (7.2 μ M) incubated with the non-specific DNA fragment (1.26 kb, 6 nM). (A) The two first rows show zoomed images (305 nm \times 305 nm) of selected DNA molecules from the corresponding (same column) representative large field AFM images (3 \times 3 μ m) on the third row. (B) Quantification of binding of EspR wt and mutants to the non-specific DNA fragment. Percentages of "relaxed" and "looped" DNA molecules are also indicated. The number of DNA molecules analyzed for each protein-DNA binding reaction is indicated. The standard error bars indicate the variation in the number of "bound" versus "unbound" DNA molecules observed in the different AFM images for a given protein-DNA binding reaction. (C) Comparison of the percentages of looped DNA structures (\pm standard error) induced by the different proteins tested.

A



B



C

