Solving the structure of the mycobacterial chromosome condensing protein Lsr2 in complex with DNA

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Lsr2 is a DNA binding protein that is highly conserved in mycobacteria and related actinomycetes and it is thought to be essential in *Mycobacterium tuberculosis*. We solved the structure of the N-terminal dimerisation domain of Lsr2 using crystallographic *ab initio* approaches[1] whereas the C-terminal DNA binding domain structure was solved by others using NMR[2]. Whilst the DNA binding mechanism is modelled based on the NMR structure, the exact mechanism of DNA binding by the entire protein is unknown. Lsr2 contains a long flexible loop between the two domains which may lead to the protein having a large range of movement, allowing it to bind DNA in a dynamic way. Current work aims to solve the structure of the fully intact Lsr2 DNA binding protein bound to a specific double-stranded DNA oligonucleotide.

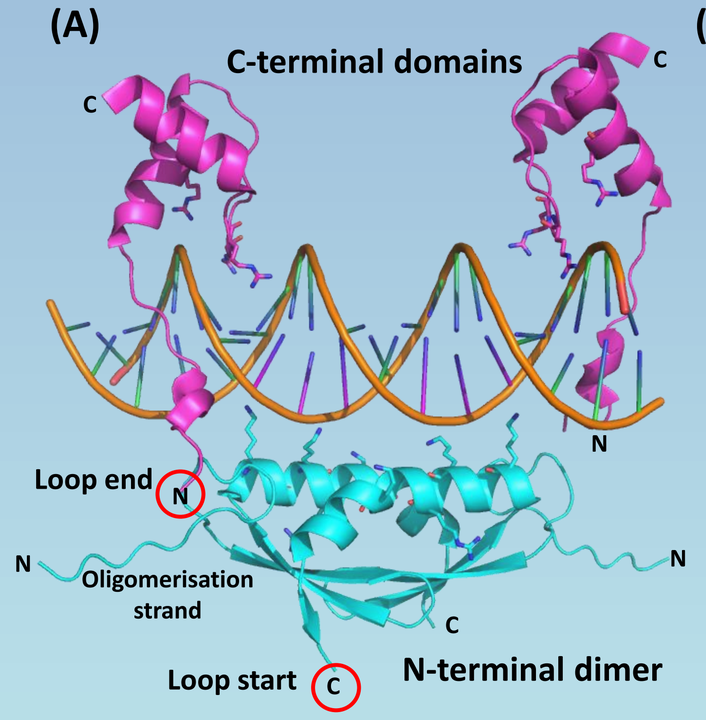


Figure 1. A proposed structure Lsr2 protein bound to DNA and evidence of DNA condensation. (A) The dimeric structure of the N-terminal dimerisation domain (blue) and two copies of the C-terminal DNA binding domain (pink) of Lsr2 were modelled onto a 20 bp DNA strand based on proposed interactions between lysine residues and the DNA backbone and arginine residues and the minor groove (for the N-terminal and C-terminal domains respectively). The "tail" of the C-terminal domain traces the major groove of the DNA based on electrostatic charge. Missing residues are proposed to form a loop between the two domains. (B) Negatively stained Lsr2/DNA complexes visualised by transmission electron microscopy show large morphological changes upon oligomerisation of Lsr2 induced by trypsin treatment. Condensed structures (arrows) are seen post digestion.

Figure 2. An N-terminal truncation of Lsr2 continues to co-purify with *E. coli* genomic DNA by IMAC but DNA-free protein will bind and purify with sequence specific dsDNA oligonucleotides. (A) IMAC purified truncLsr2 co-purifies with *E. coli* DNA (with greater amounts of DNA under autoinduction), which is removed via size exclusion chromatography (SEC) with a 2M NaCl buffer. Oligos with * in table 1 were bound to protein produced via autoinduction. (B) Lsr2 in standard low NaCl buffer is incubated with dsDNA oligonucleotides and purified via SEC for crystallography trials.

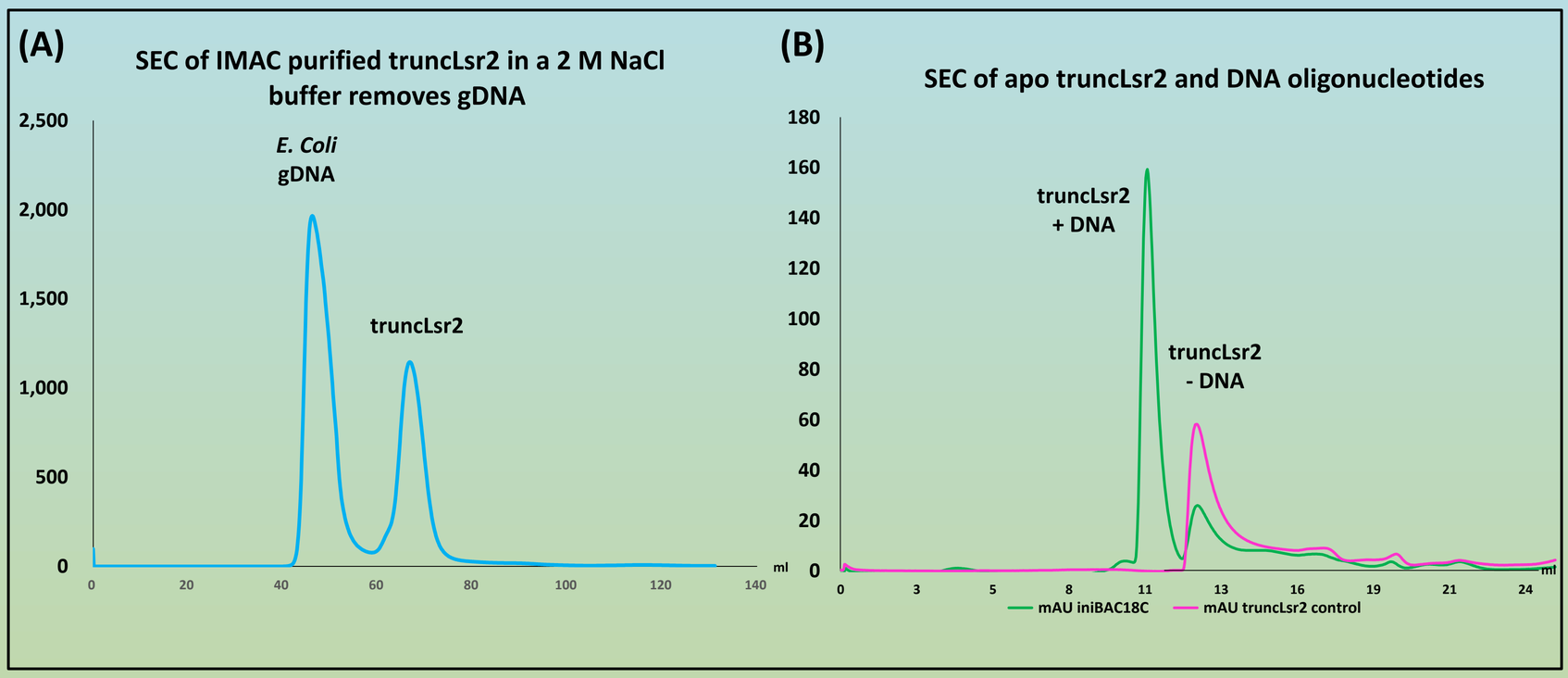
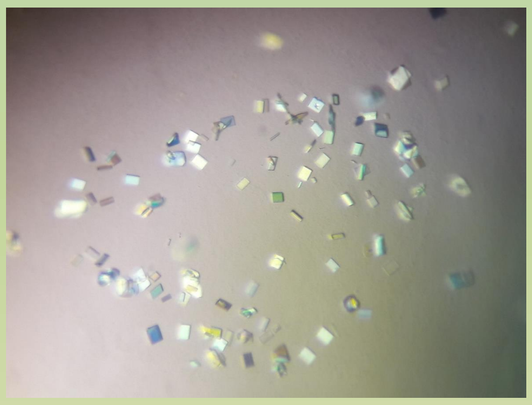


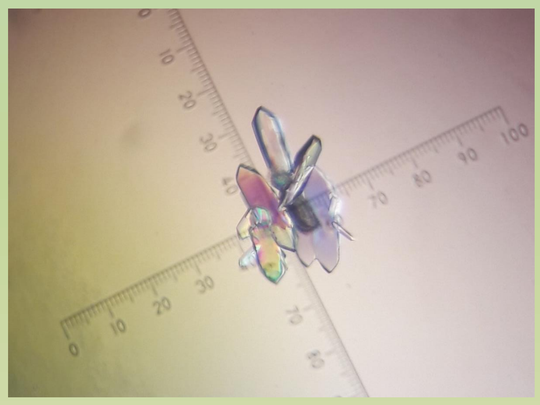
Table 1. DNA oligos were bound to truncLsr2 to aid crystallisation.

Oligo Name	Features	Outcome
C42	"curved", 42 bp, strings of AAAAA	unsuccessful
NC42	"non-curved", 42 bp mixed ATC	unsuccessful
iniBACp10	Based on promoter for iniBAC operon, 10 bp	unsuccessful
iniBACp20	Based on promoter for iniBAC operon, 20 bp	Xtals too small to test
Lsr2palin18	Adjusted iniBACp20 to be palindromic, 18 bp	unsuccessful
Lsr2palin20	Adjusted iniBACp20 to be palindromic, 20 bp	unsuccessful
Lsr2palin22	Adjusted iniBACp20 to be palindromic, 22 bp	unsuccessful
iniBACp18A	Trimmed 2 bp from iniBACp20 upstream	unsuccessful
iniBACp18B	Trimmed 2 bp from iniBACp20 downstream	unsuccessful
iniBACp18C	Trimmed 2bp from iniBACp20 1 bp each end	Xtals nondiffracting
Gilston	Pseudo-palindromic, 33 bp, 75% AT, G contacts	Xtals not replicated
Rajasekar	19 bp, 60% AT, Guanine contacts	Xtals nondiffracting
tLsr2DNase	Co-purified *E. coli* DNA trimmed with DNase	Xtals nondiffracting
Cui	19 bp, 63% AT, Guanine contacts	Diffraction to 6.3 Å **(A)**
Cui20*	Alteration of Cui, added T/A overhang, 20 bp	Diffraction to 2.7 Å **(B)**
Cuipalin20	Alteration of Cui, made palindromic, 20 bp	Xtals not replicated
Cui18bpA*	Removed 1bp from Cui upstream	Xtals nondiffracting
Cui18bpB*	Removed 1bp from Cui downstream	Xtals poorly formed
Kurthkoti	19 bp, 58% AT, operator, no structure in literature	Binds to protein
Alland	19 bp, 58% AT, operator, no structure in literature	Binds to protein

(A)



(B)



To aid in the crystallisation of Lsr2, the protein was truncated to prevent it from undergoing oligomerisation and stabilised by binding it to double-stranded DNA. Both methods were designed to produce a uniform population of protein for improving crystallographic success. Initial testing of binding different oligonucleotides of different lengths to Lsr2 revealed that 18-20 bp in length was optimal and could generate crystals. Literature searching for DNA oligonucleotides that have been used in crystallography helped extend the search for the appropriate DNA sequence. To date, the oligonucleotide "Cui20" has generated crystals that diffracted to 2.7 Å but this work is ongoing.

1. Summers, E., K. Meindl, I. Uson, A. Mitra, M. Radjainia, R. Colangeli, D. Alland and V. Arcus (2012). "*The structure of the oligomerization domain of Lsr2 from Mycobacterium tuberculosis reveals a mechanism for chromosome organization and protection*." PLoS ONE **7**(6): e38542.
2. Gordon, B. R. G., Y. Li, L. Wang, A. Sintsova, H. van Bakel, S. Tian, W. W. Navarre, B. Xia and J. Liu (2010). "*Lsr2 is a nucleoid-associated protein that targets AT-rich sequences and virulence genes in Mycobacterium tuberculosis*." Proceedings of the National Academy of Sciences **107**(11): 5154-5159.