Structural characterization of Nuclear Factor I X in complex with its target DNA

Tiberi M, Cavinato M, Chaves-Sanjuan A, Messina G, Gourlay LJ, Nardini M, Email: michele.tiberi@unimi.it
Department of Biosciences, University of Milan, via Celoria 26, 20133 Milano (Italy)

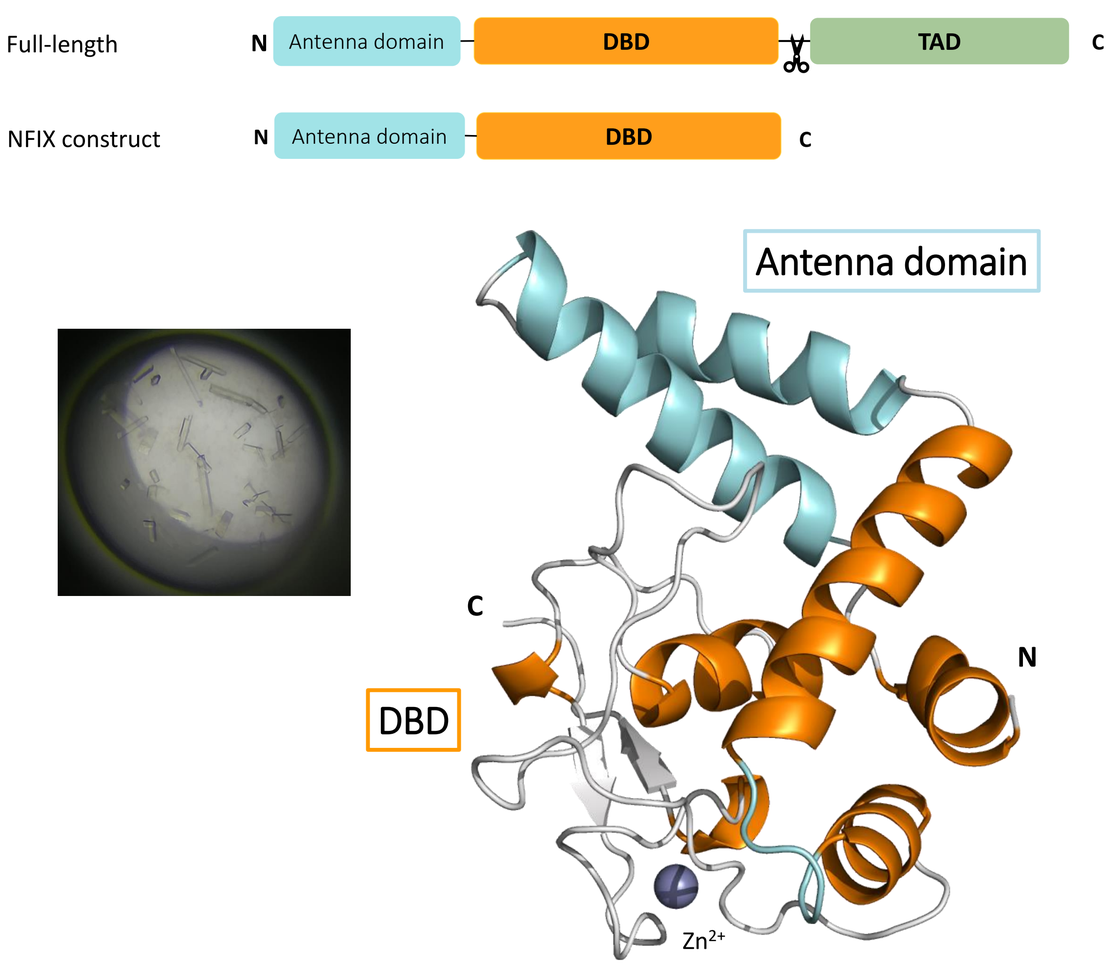
INTRODUCTION

Nuclear Factor I X (NFIX) is a transcription factor belonging to the Nuclear Factor I family. It binds as a dimer a palindromic DNA sequence [1] and it is involved in several processes and pathologies. In the context of muscular dystrophy, skeletal muscle fibers lacking NFIX have been shown to slow down the dystrophic phenotype [2]. Moreover, several mutations falling into the DNA-binding domain (DBD) of NFIX lead to the Malan syndrome, an overgrowth syndrome characterized by intellectual disability [3]. Here we present the X-ray structure of NFIX-DBD and a proposed model for protein-DNA interaction, that is consistent with the negative effect on NFIX DNA-binding due to the DBD mutations present in Malan syndrome patients.

RESULTS

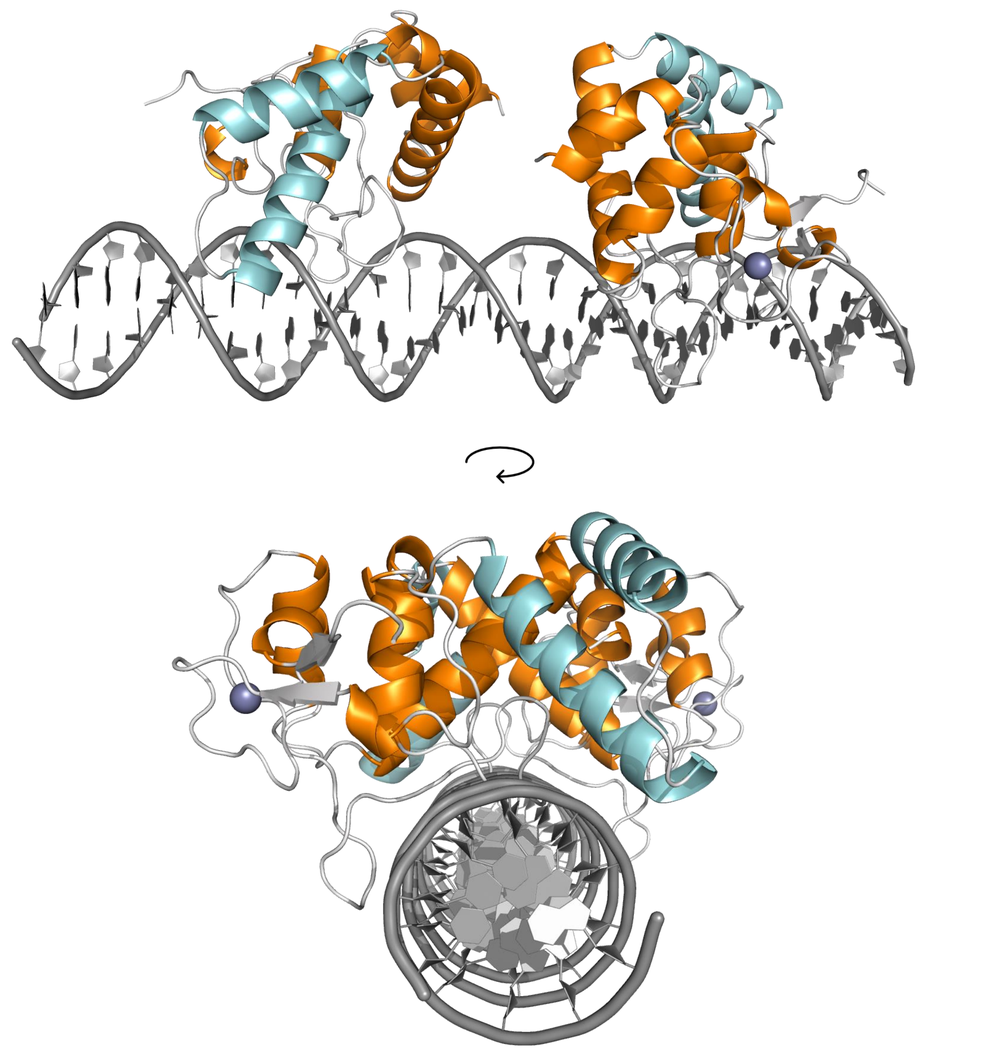
1. Crystallization and X-ray structure

We designed a truncated construct of NFIX to exclude the intrinsically disordered C-terminal transactivation domain (TAD) and its structure was solved at 2.7 Å resolution by X-ray diffraction (in collaboration with the ELETTRA synchrotron team). However, **the structure of NFIX in complex with its target DNA is still unknown.**



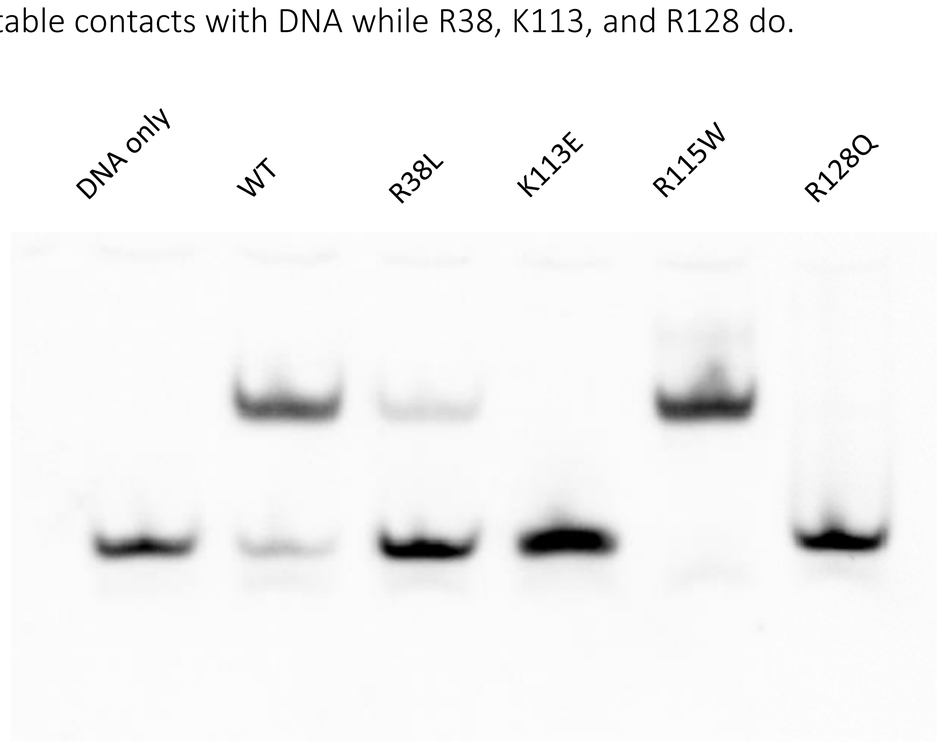
2. NFIX-DNA binding model

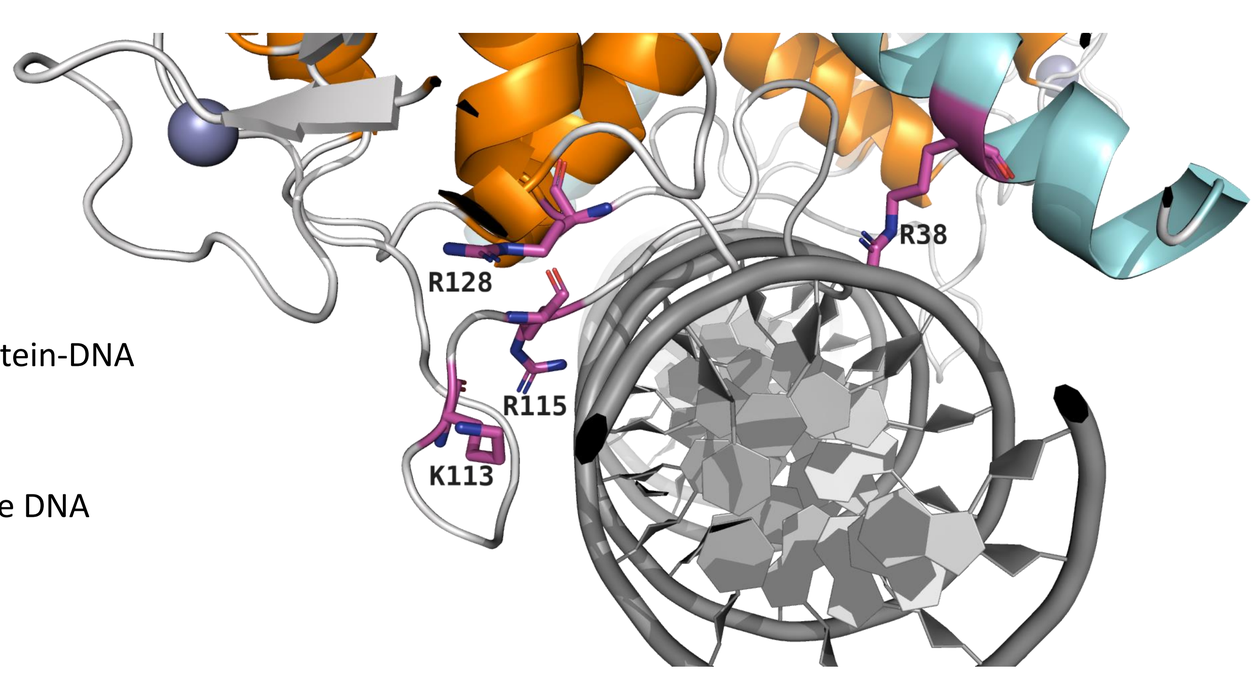
Exploiting the structural homology of NFIX with a transcription factor belonging to the Smad family, we built a predictive **model for NFIX-DNA interaction** starting from a structural alignment of NFIX and a Smad protein bound to its target DNA.



3. DNA-binding assay

We have successfully expressed and purified four Malan syndrome mutants (R38L, K113E, R115W and R128Q). The effect of the mutations on NFIX-DNA binding was evaluated by Electrophoretic Mobility Shift Assay (EMSA). **Three of these mutants showed a reduced or abolished DNA-binding ability**, consistently with the position of the mutated residues in our NFIX-DNA model. Preliminary MD simulations (in collaboration with Elisabetta Moroni, CNR-Milano) confirm that R115 doesn't provide stable contacts with DNA while R38, K113, and R128 do.





CONCLUSIONS AND PERSPECTIVES

We obtained important information about the NFIX-DBD structure and the structural homology with Smad proteins allowed us to build a model for protein-DNA interaction. However, experimental data on the NFIX-DNA complex will provide essential knowledge on the molecular mechanisms of DNA-binding and for the rational design of drugs that modulate NFIX function. Indeed, compounds that inhibit NFIX function may be used in muscular dystrophy treatments, while drugs that stabilize the protein-DNA complex may find an application in Malan syndrome. With this aim, we are currently working on the resolution of the atomic structure of NFIX in complex with its target DNA (by SAXS and X-ray crystallography).

[1] R.M. Gronostajski, Nucleic Acids Res. (1986), 14, 9117. [2] M. Piper, R. Gronostajski, G. Messina, Trends Cell Biol. (2019), 29, 20. [3] Y. Lu, P.F. Chong, R. Kira, T. Seto, Y. Ondo, K. Shimojima, T. Yamamoto, J. Pediatr. Genet. (2017), 6, 234.