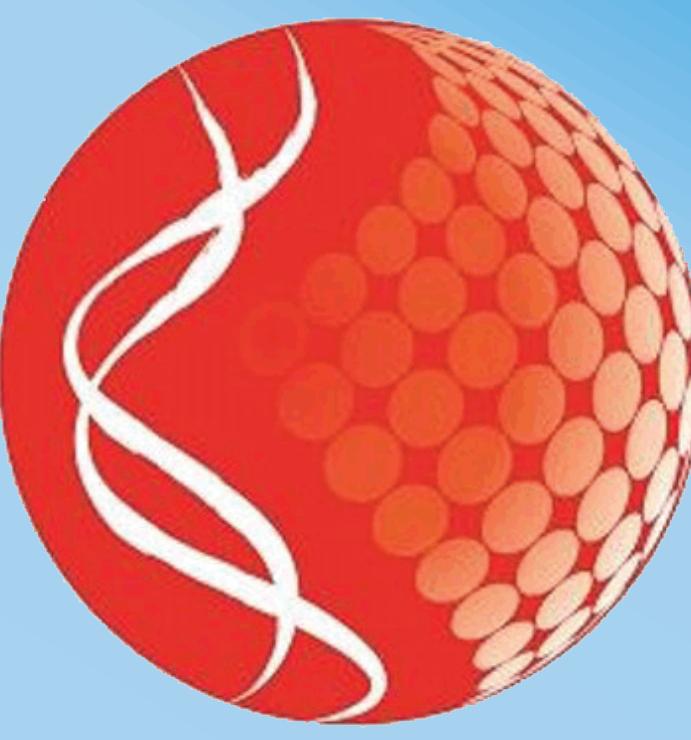




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Structural Insights into the DNA Binding Activity of the Ferric Uptake Regulator in *Campylobacter jejuni*



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Introduction

- Campylobacter jejuni* is responsible for campylobacteriosis, a common cause of gastroenteritis in humans [1]. Campylobacteriosis has also been linked with the pathogenesis or exacerbation of debilitating autoimmune conditions [2].
- Iron is vital to many cellular processes in *C. jejuni*, including energy metabolism, DNA biosynthesis, virulence, and oxidative stress response [3].
- The transcription factor Ferric Uptake Regulator (Fur) tightly regulates the uptake of iron by the cell to maintain adequate intracellular iron levels and limit the formation of deleterious reactive oxygen species [3].
- Fur in *C. jejuni* (CjFur) responds to the varying bioavailability of iron in its environment and engages in four major regulation modes [4]:

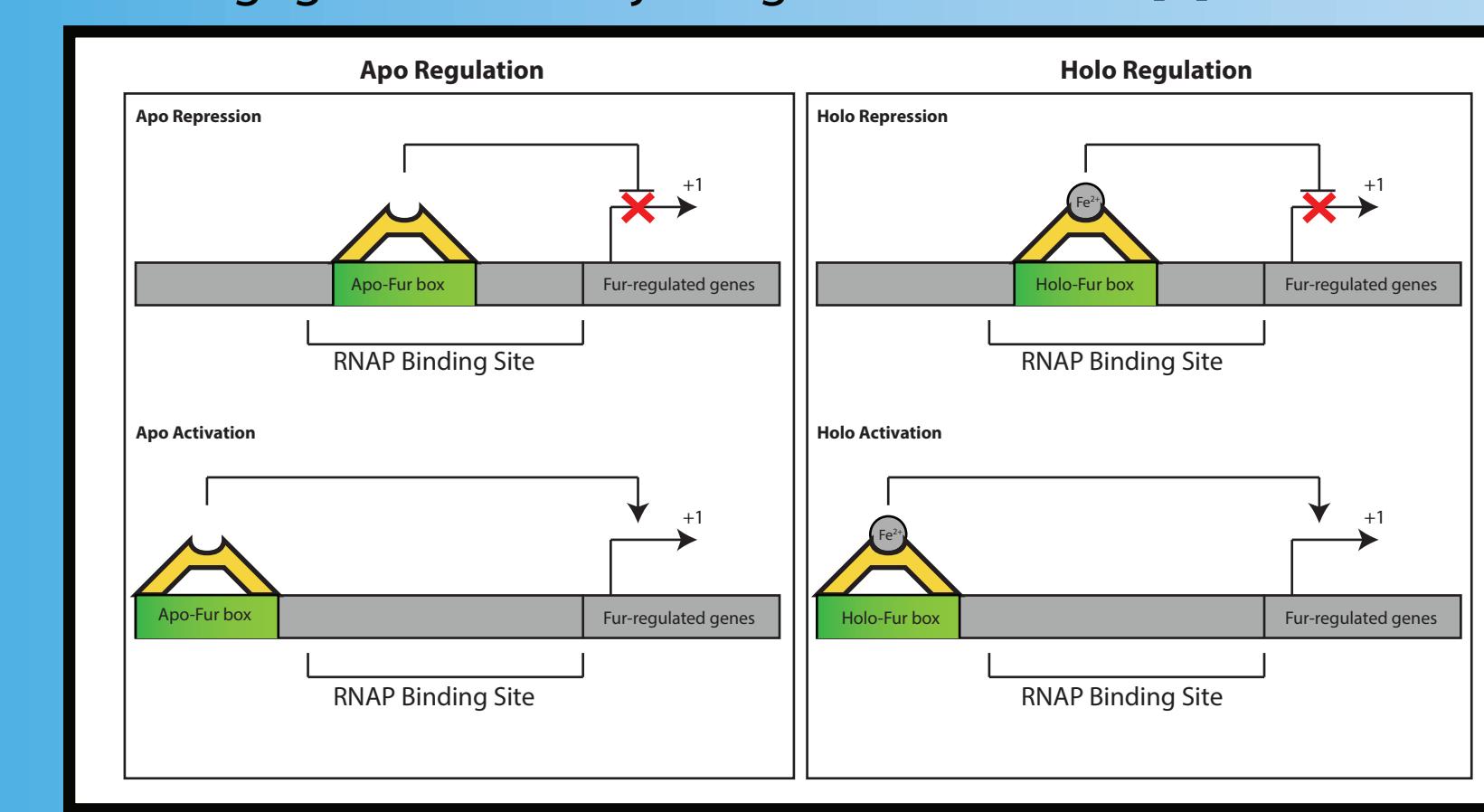


Figure 1. The major regulation modes employed by CjFur. CjFur may activate or repress expression of mRNA under iron deplete conditions (apo-regulation), or under iron replete conditions (holo-regulation).

- Apo-CjFur folds into a DNA binding domain (DBD) and a dimerization domain (DD), and dimerizes to achieve the canonical V-shaped conformation characteristic to holo-Fur proteins [4].

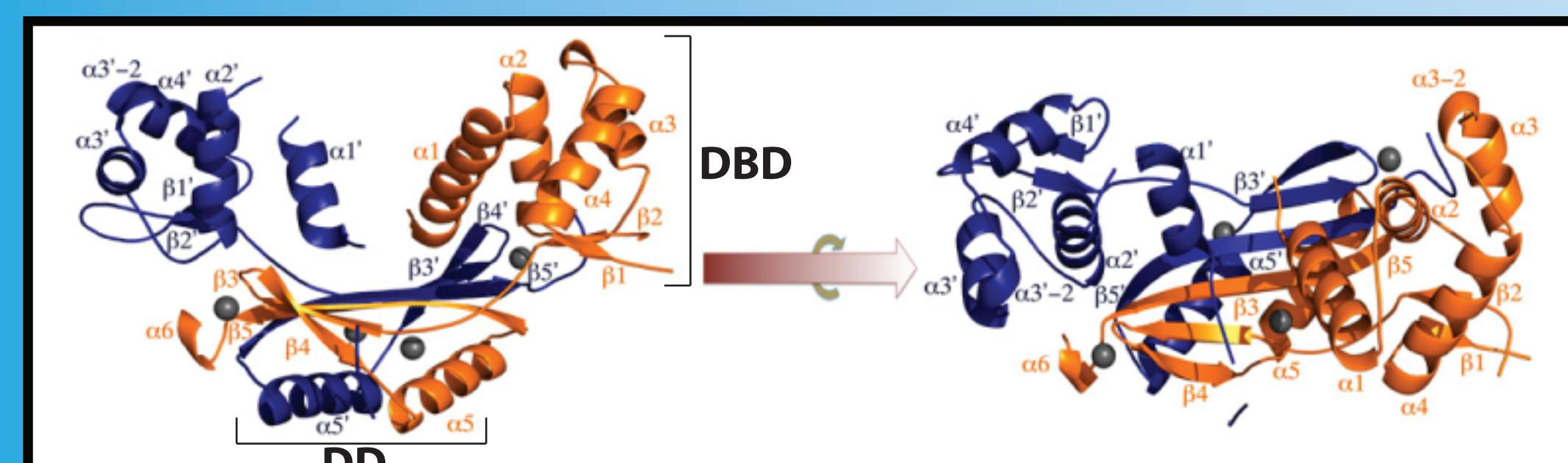


Figure 2. The crystal structure of apo-CjFur. Shown are orthogonal views of apo-CjFur. The protomers are in blue and orange, and the structural zinc atoms are in grey.

- Interestingly, apo-CjFur, in contrast with its holo homologs, positions its $\alpha 1$ helix towards the inside instead of the outside.
- Structural alignment of apo-CjFur with its closest homolog, holo-HpFur, has revealed a significant difference in the conformation of the hinge region connecting the DBD to the DD [4,5].

Hypothesis

- Our previous structural studies revealed that the remnant of the SUMO tag tightly associates with the DD domain which could "lock" the structure of the apo-CjFur in a conformation different than the other structurally determined Holo-FUR. In this study, we hypothesized that the remnant of the tag does not influence the orientation of FUR DBD domain.

Objectives

- To confirm our hypothesis we engaged in structural and mutational studies *in vitro*. We specifically sought to fulfill two objectives:
- Crystallize and solve the crystal structure of apo-CjFur using a construct with a different tagging approach.
 - Characterize holo-CjFur mutants and their DNA binding activity

Overexpression and Purification of CjFur

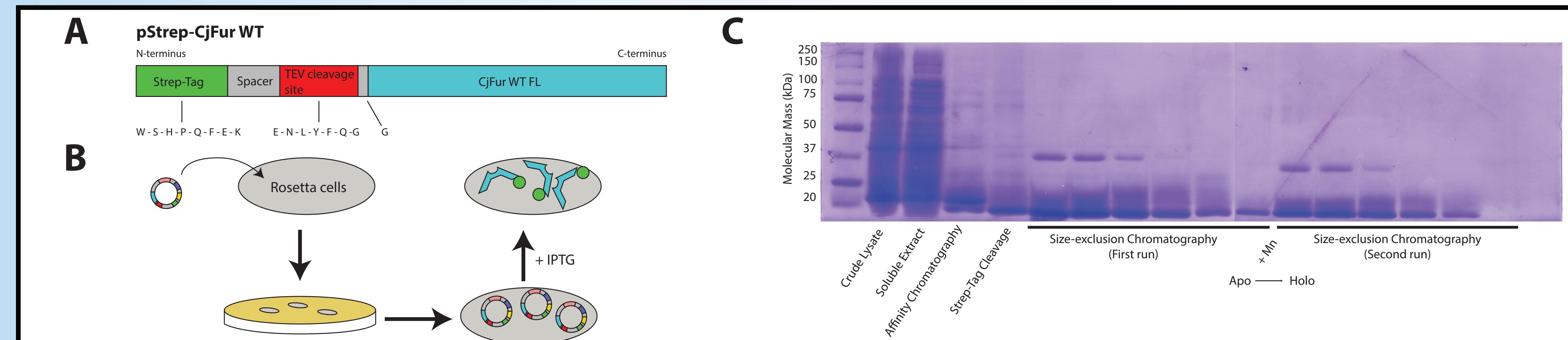


Figure 3. The purification protocol for CjFur yields pure protein amenable to crystallization experiments. (A) The optimized CjFur WT sequence was inserted in a pStrep vector to express a construct comprising a Strep-Tag, a TEV protease cleavage site and CjFur WT. (B) The protein construct was overexpressed in *E. coli* Rosetta cells. (C) Aliquots of the protein sample at the different steps of the purification procedure were run on an SDS-PAGE gel to assess protein purity and yield.

A New Apo-CjFur Crystal Structure

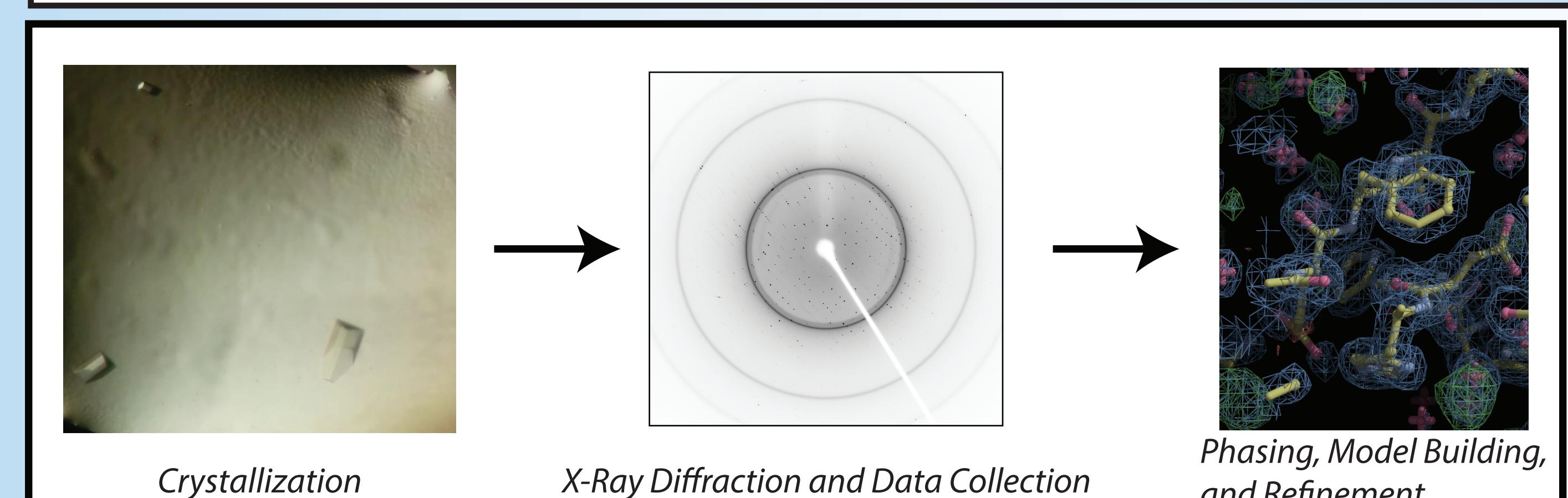


Figure 4. Crystal structure of Apo-CjFur. CjFur was purified and crystals were grown in a mother liquor containing 0.1 M Bis-Tris pH 5.5, 0.25M MgFor, 21% PEG3350. The crystals were harvested, cryoprotected, and exposed to X-Rays. The phasing was done using molecular replacement. The model was built with Coot, and refined with Buster.

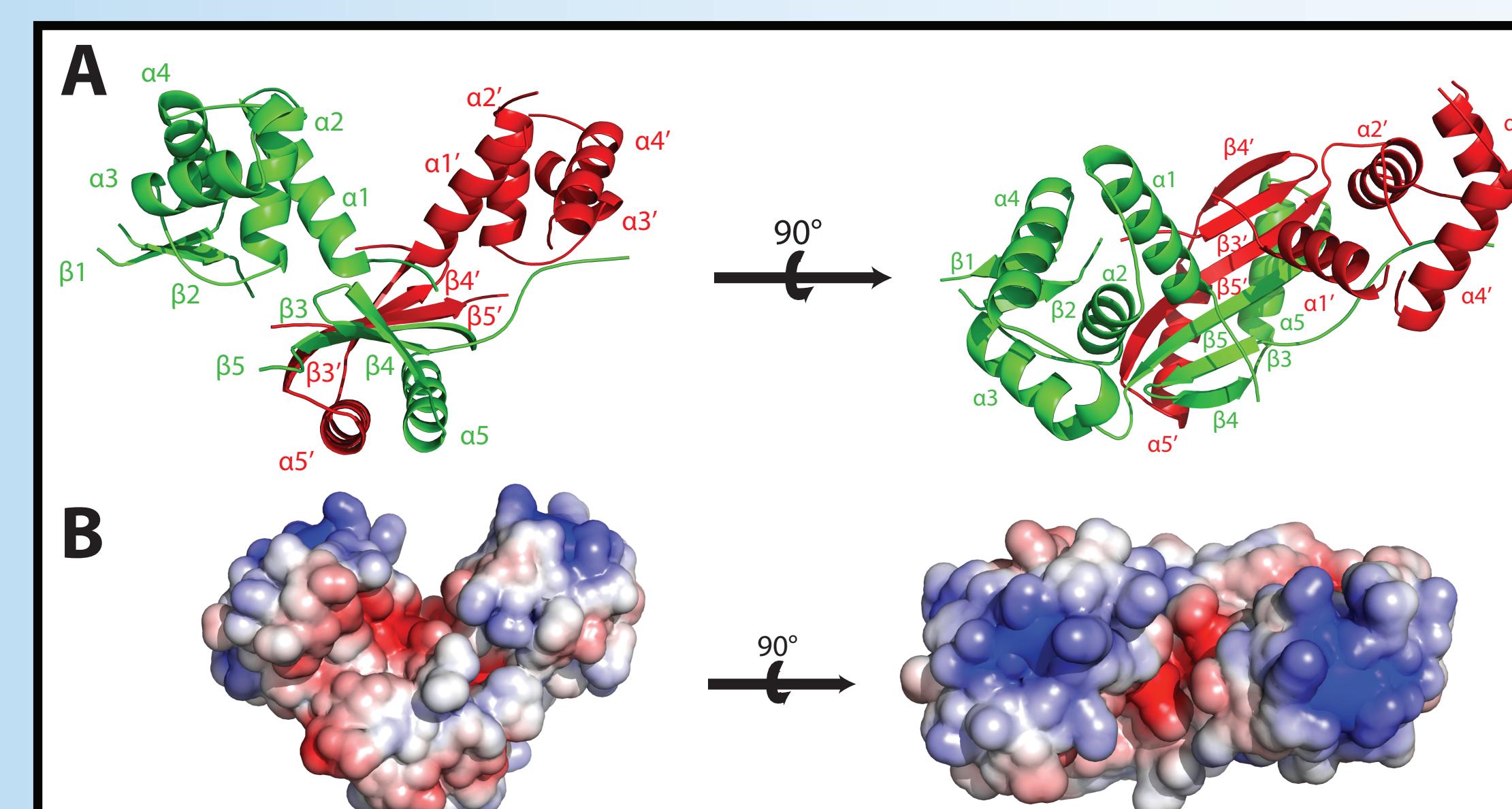


Figure 5. CjFur was crystallized in its apo conformation. (A) Shown are orthogonal views of the new apo-CjFur crystal structure. Secondary structures are labeled. (Structural zinc atoms not shown) (B) Electrostatic surface potential ($-5 \text{ k}_\text{b} \text{Te}^{-1}$ to $5 \text{ k}_\text{b} \text{Te}^{-1}$) of the newly obtained crystal structure of apo-CjFur. Positively charged regions are in blue, whereas negatively charged regions are in red.

The Holo-CjFur-DNA Complex

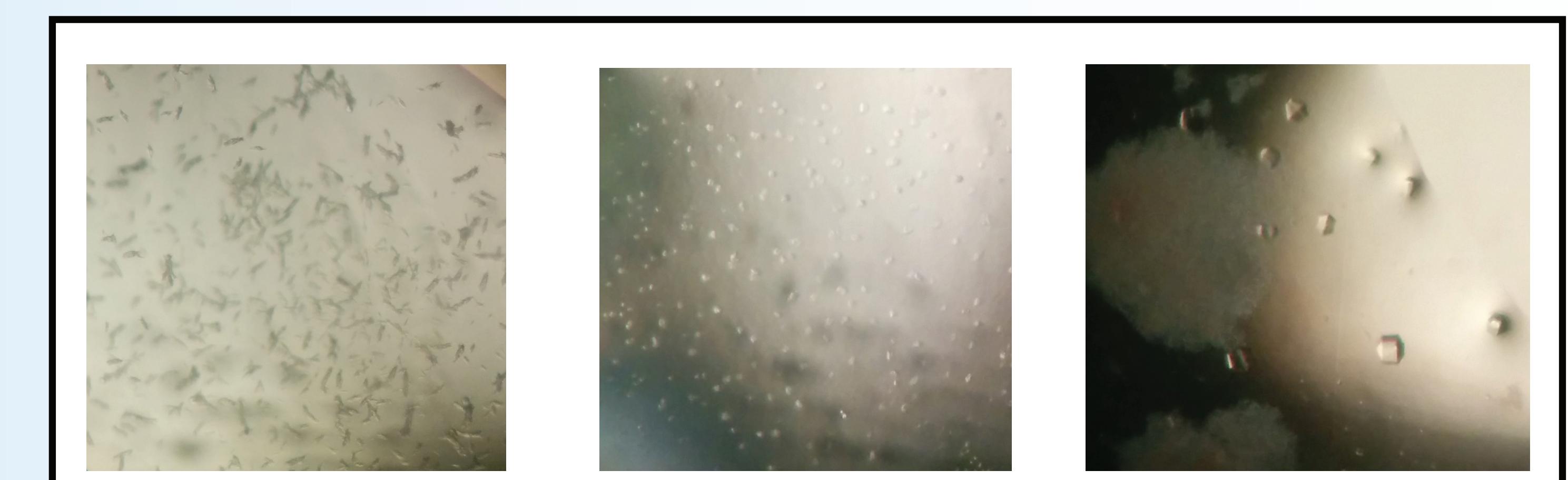


Figure 7. Crystallization attempts for the holo-CjFur-DNA complex.

A library of oligonucleotides (34) corresponding to the Fur-box in the promoter of the *katA* gene were screened in an attempt to crystallize the Holo-CjFur-DNA complex. The length of the oligonucleotides was varied (15-41bp), as well as the nature of its ends (blunt ends and sticky ends) to see whether these parameters could facilitate the crystallization of the complex through Watson-Crick complementary interactions between adjacent DNA molecules.

In vitro Characterization of the CjFur R14E and K17E Mutants

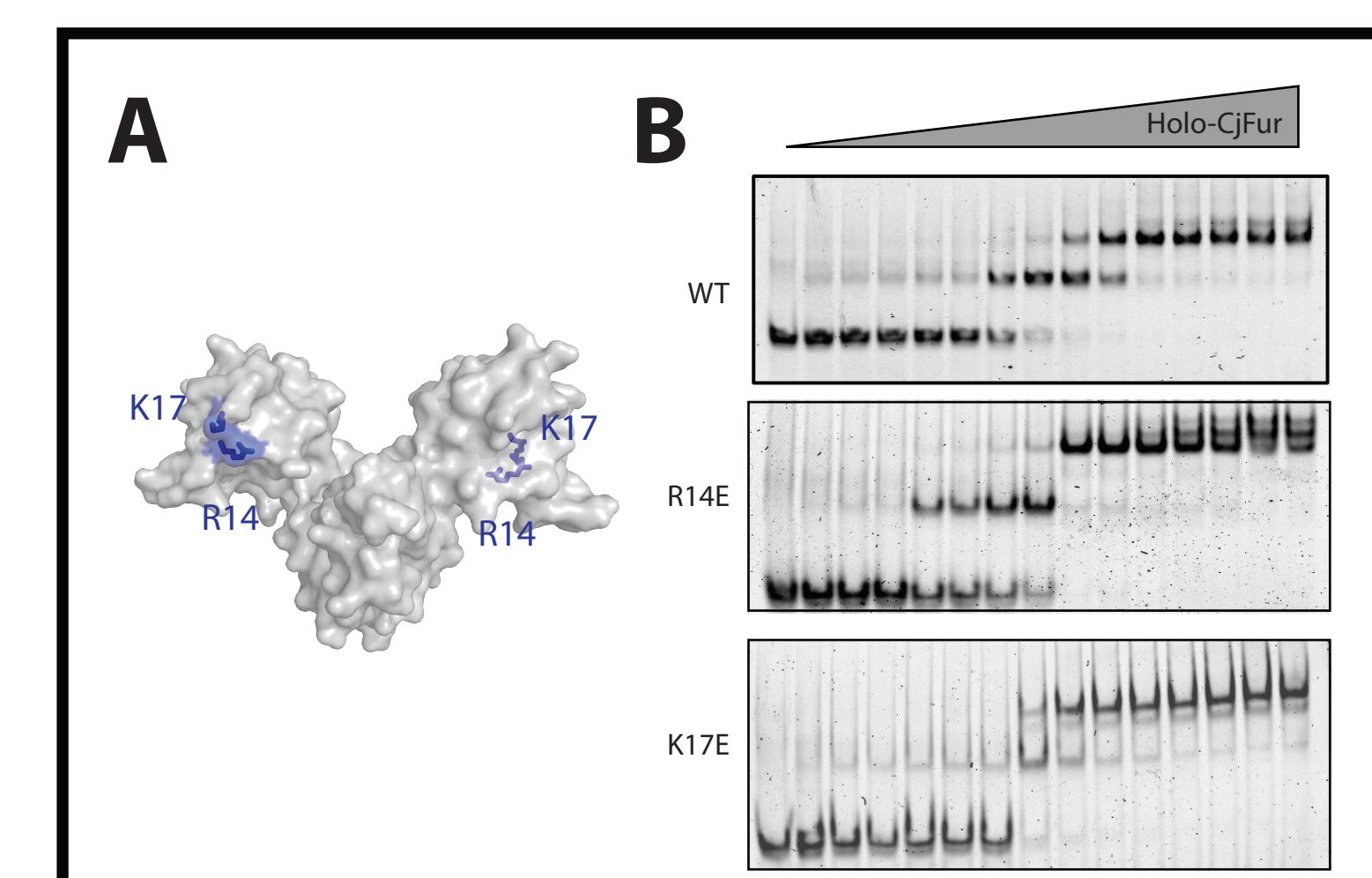


Figure 8. Characterization of the CjFur R14E and K17E mutants by EMSA. (A) The residues highlighted in blue were mutated to Glu to evaluate their role in the DNA binding activity of holo-CjFur with DNA. (B) A Cy5 labeled probe corresponding to a 60 bp fragment from the *katA* promoter region (1 nM) was titrated with increasing concentrations (0-100 nM) of holo-CjFur WT (top), R14E (middle) and K17E (bottom) and run on a native polyacrylamide gel.

Conclusions

- The remnant of the tag does not influence the conformation of FUR DBD
- The resolution of apo-CjFur using a new protein construct provides additional evidence supporting the existence of apo-CjFur and the apo-regulation mode.
- Residues R14 and K17 do not play a significant role in the DNA binding activity of holo-CjFur in the conditions tested.

Future Work

- Mutate bulky, charged residues (Lys and Glu) on the CjFur surface to reduce the entropic barrier associated with the crystal packing of holo-CjFur
- Screen additional oligonucleotides to crystallize the holo-CjFur-DNA complex

Acknowledgments

I would like to express my gratitude to:

- Prof. Jean-François Couture, for his trust and guidance throughout the course of this project
- Sabina Sarvan, for her scientific mentoring, but also for her exceptional generosity, patience and support
- The Couture Lab members, for their help, the good laughs, and their positive attitude
- Our collaborator Joseph Brunelle
- The following funding agencies:

Fonds de recherche
Santé
Québec

Canada Foundation for Innovation
Fondation canadienne pour l'innovation

CIHR
IRSC
Canadian Institutes of
Health Research
Instituts de recherche
en santé du Canada

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