Structural study of transcriptional factor CifR regulating a virulence factor from *Pseudomonas aeruginosa*

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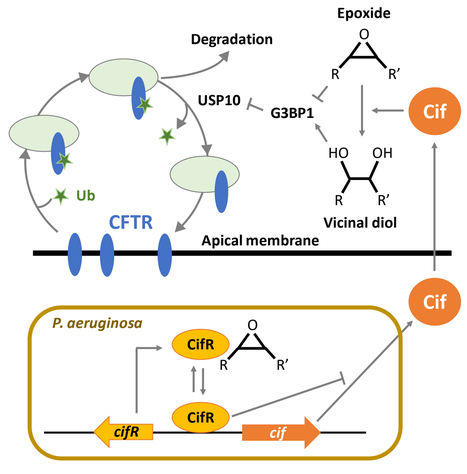
Background:

Pseudomonas aeruginosa is an opportunistic pathogen and a leading cause of airway infection, particularly in patients with underlying lung disease. For example, *P. aeruginosa* colonizes >85% patient with Cystic Fibrosis and is a major contributor to respiratory failure in most of these patients.

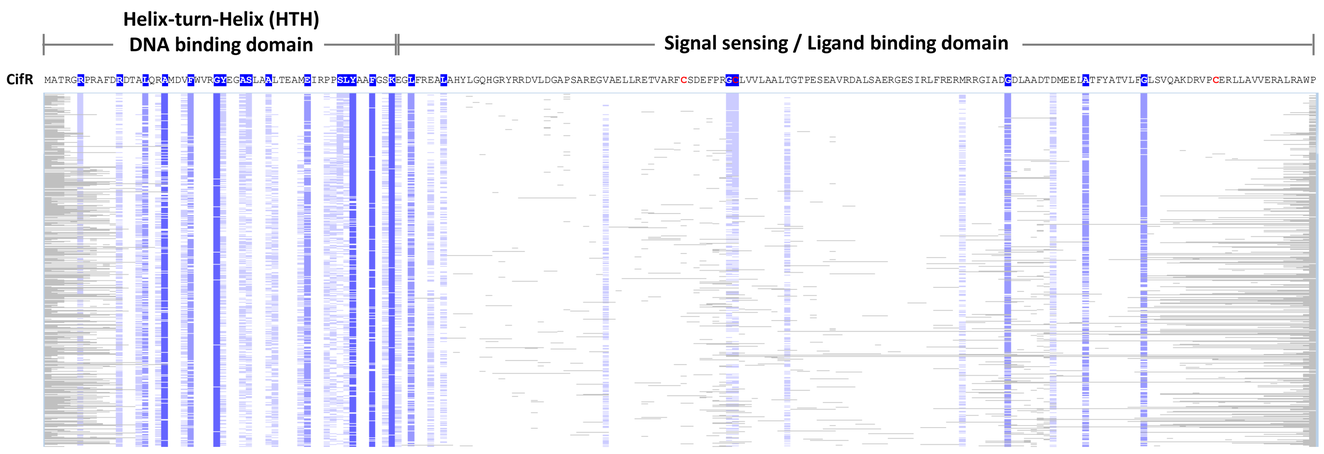
The previous studies in the lab have uncovered a mechanistically **novel *P. aeruginosa* virulence factor, the CFTR inhibitory factor (Cif),** an **epoxide hydrolase (EH) enzymes.** It reduces the cell-surface abundance of the cystic fibrosis transmembrane conductance regulator (CFTR) in airway epithelial cells via its epoxide-hydrolyzing activity. Cif can thus subvert mucociliary clearance, a major early line of defense against colonization by respiratory pathogens. Cif also sabotages pro-resolving signaling pathways, supporting a hyperinflammatory airway milieu.

In *P. aeruginosa*, Cif expression is regulated by a **TetR family transcriptional repressor CifR**. The expression of *cif* gene is repressed upon CifR binding on *cif* promotor. Previous studies in the lab have shown that CifR-mediated repression of *cif* gene **expression is relieved by addition of epoxides** *in vivo* and that **CifR-DNA binding activity is disrupted by epoxide** *in Vitro.* This makes CifR the **first reported epoxide-sensitive** bacterial transcriptional regulator.

The study here revealed the first molecular structure of CifR-DNA complex which leads to understand the regulation mechanism of CifR on the expression of the virulence gene *cif*. This provides fundamental support for using CifR as a new therapeutic target and for developing potential drugs that may complement existing antibiotic regimes.

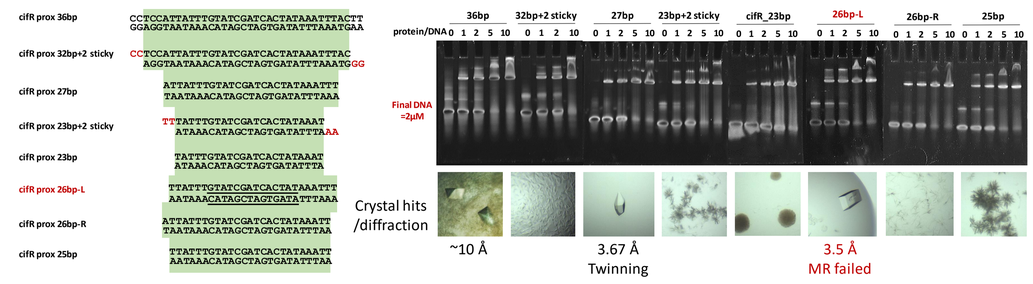


(1) CifR protein domain organization and sequence conservation



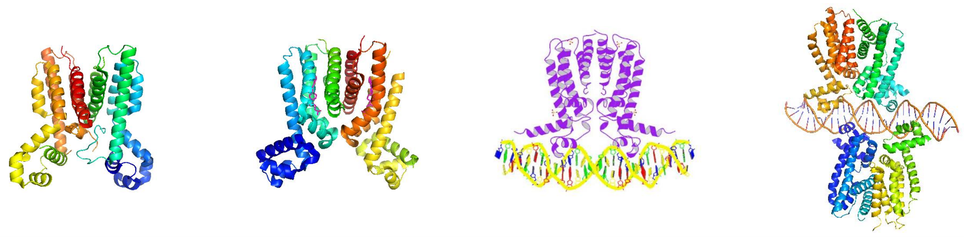
Jpred alignment using PSI-BLAST (Position-Specific Iterative Basic Local Alignment Search Tool). 938 Sequences various from 100 aa to 196 aa have been used in the alignment.

(2) DNA screening for best crystallization score



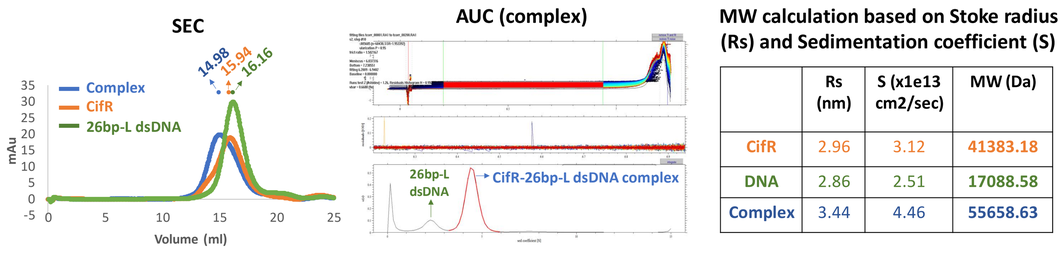
(3) Structure of CifR homologs

Putative TetR-family member | EthR2- BDM41420 | DesT-DNA | QacR-DNA



Organism	*Rhodococcus jostii*	*Mycobacterium tuberculosis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Identity to CifR	41%	17%	26%	31%
PDB id	2i10	5icj	3lsr	1jt0
Diffraction resolution	2.05Å	2.4Å	2.55Å	2.9Å
Phasing method	Single-wavelength anomalous dispersion (SAD)	Iodine-Single wavelength Anomalous Dispersion method (I-SAD)	Single-wavelength anomalous dispersion (SAD)	Multiple Isomorphous Replacement (MIR)

(4) Stoichiometry of CifR-DNA complex: 1 dimer + 1 dsDNA

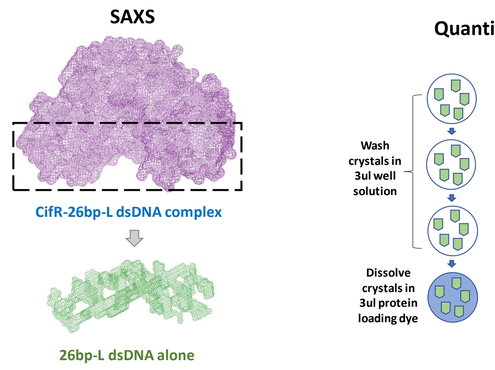


MW calculation based on Stoke radius (Rs) and Sedimentation coefficient (S)

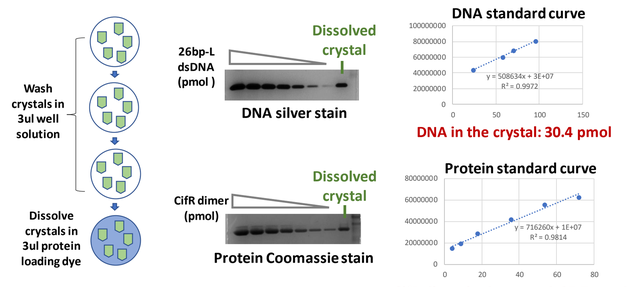
	Rs (nm)	S (x1e13 cm2/sec)	MW (Da)
CifR	2.96	3.12	41383.18
DNA	2.86	2.51	17088.58
Complex	3.44	4.46	55658.63

MW = S(RT)/D(1-vρ)= ((6*pi*eta*Rs)/KT)*S(RT)/(1-vρ)= (6*pi*R/K) *Rs*S*eta /(1-vρ)

Svedberg equation | Stokes–Einstein equation | Gas constant / Boltzmann constant

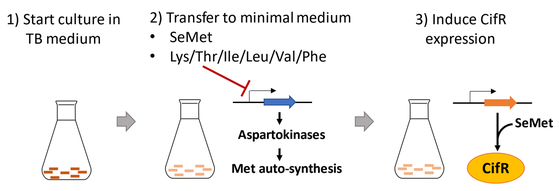


Quantification of protein and DNA in the complex crystals

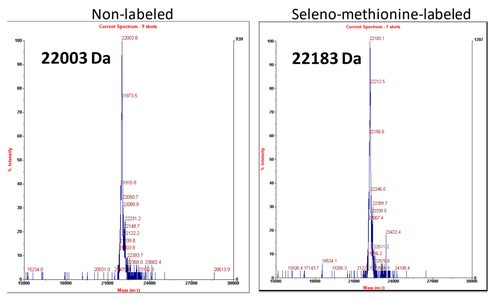


(5) Structure determination of CifR-DNA complex

Seleno-Methionine incorporation in CifR

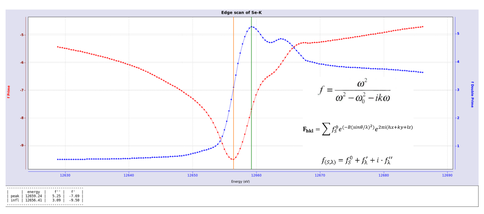


Verification of SeMet labeling by MALDI TOF-MS

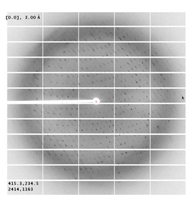


Approximately 4 out of 5 methionine have been replaced by SeMet!

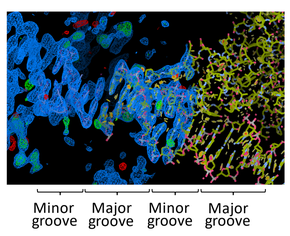
Multi-wavelength Anomalous Dispersion Edge Scan



Diffraction pattern



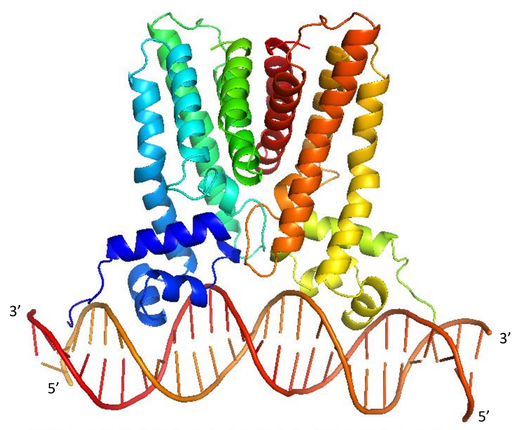
Lattice packing is determined by DNA in the crystal



Data collection and refinement statistic

	Peak	Inflection	Remote	Native dataset
Data collection				
Space group		P21 21 2		
Unit cell dimension a, b, c α, β, γ (°)		63.517 165.569 81.529 90.000 90.000 90.000		63.453 165.548 81.349 90.000 90.000 90.000
Resolution (Å)	20.0-3.0	20.0-3.0	20.0-3.0	20.0-2.5
R-merge:	133.8%	114.2%	136.2%	176.3%
I/sigma	1.55	1.58	1.30	1.6
Completeness	100%	100%	100%	100%
No. reflection	376555	374297	374777	431738
Refinement				
R-work/R-free		0.2674/0.3168		0.2316/0.2619
Ramachandran outliers		3.20%		0.53%
Rotamer outliers		0%		7.09%
Water		0		51

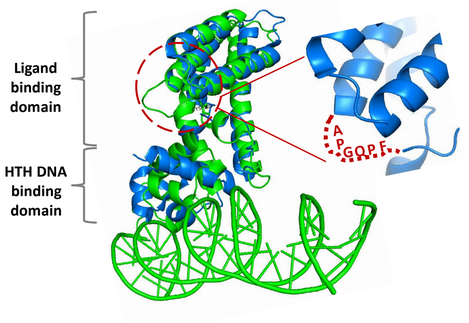
Structure of CifR-26bp-L dsDNA



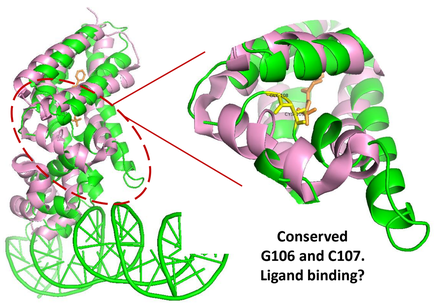
(Strand in orange) 5′- **TTATTTGTATCGATCACTATAAATTT** -3′

(6) Conformational change between DNA-bound state and ligand-bound state?

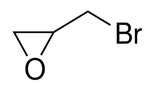
Structure alignment of CifR-2i10 (CifR homolog, apo)



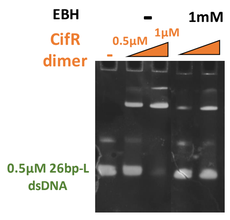
Structure alignment of CifR-5icj (CifR homolog, ligand-bound)



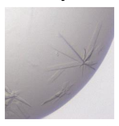
Epibromohydrin (EBH)



EMSA

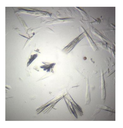


CifR-EBH crystallization



Diffracted at 4 Å
Space group: C121
Unit cell dimension:
a, b, c : 141.1 43.4 74.2
α, β, γ (°): 90.0 92.6 90.0
MR failed!

CifR (SeMet)-EBH crystalization



Working on it…

(7) High throughput screening for potential drugs