Tools for the Characterization and Manipulation of Reductive Dehalogenases for Bioremediation of Chlorinated Solvents

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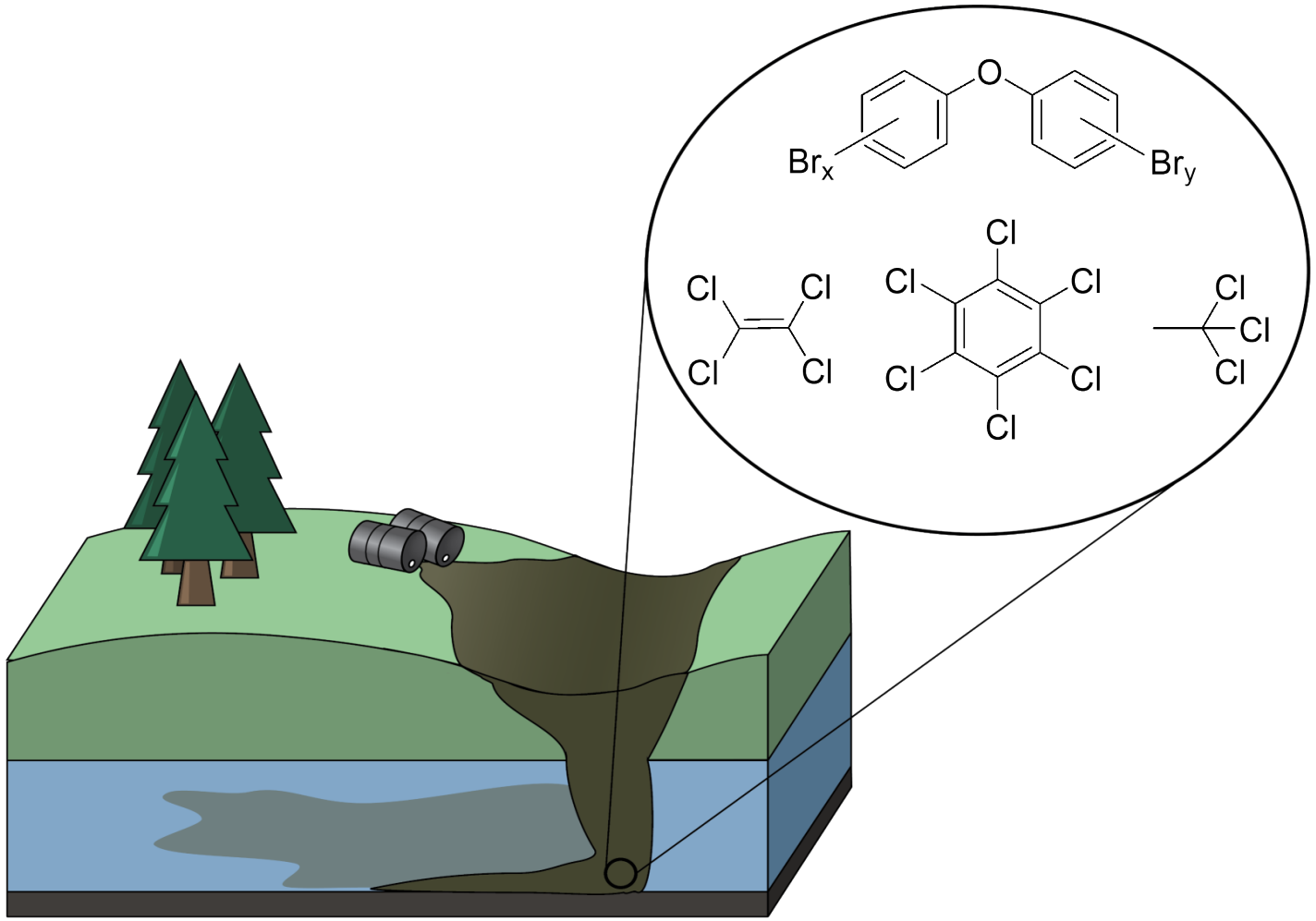
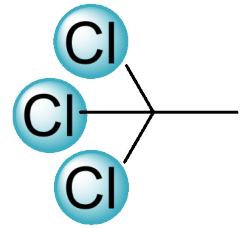
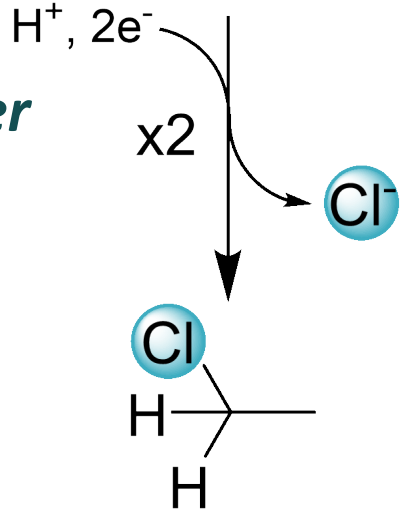
Organohalide Bioremediation





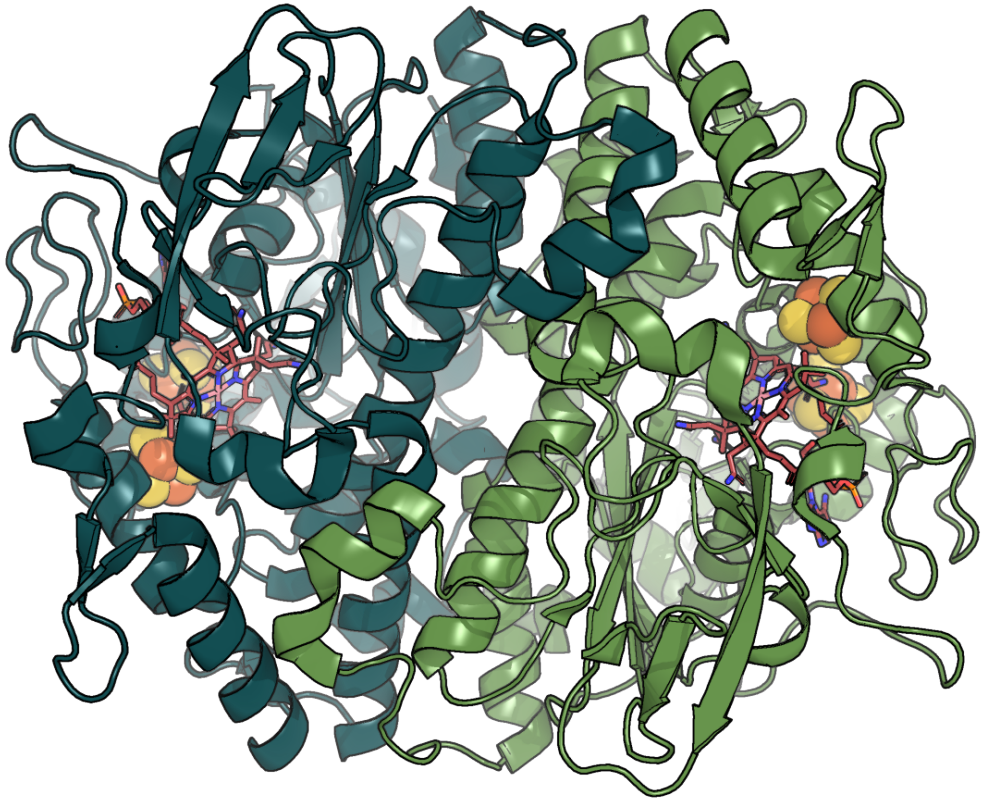
1,1,1-Trichloroethane

Dehalobacter

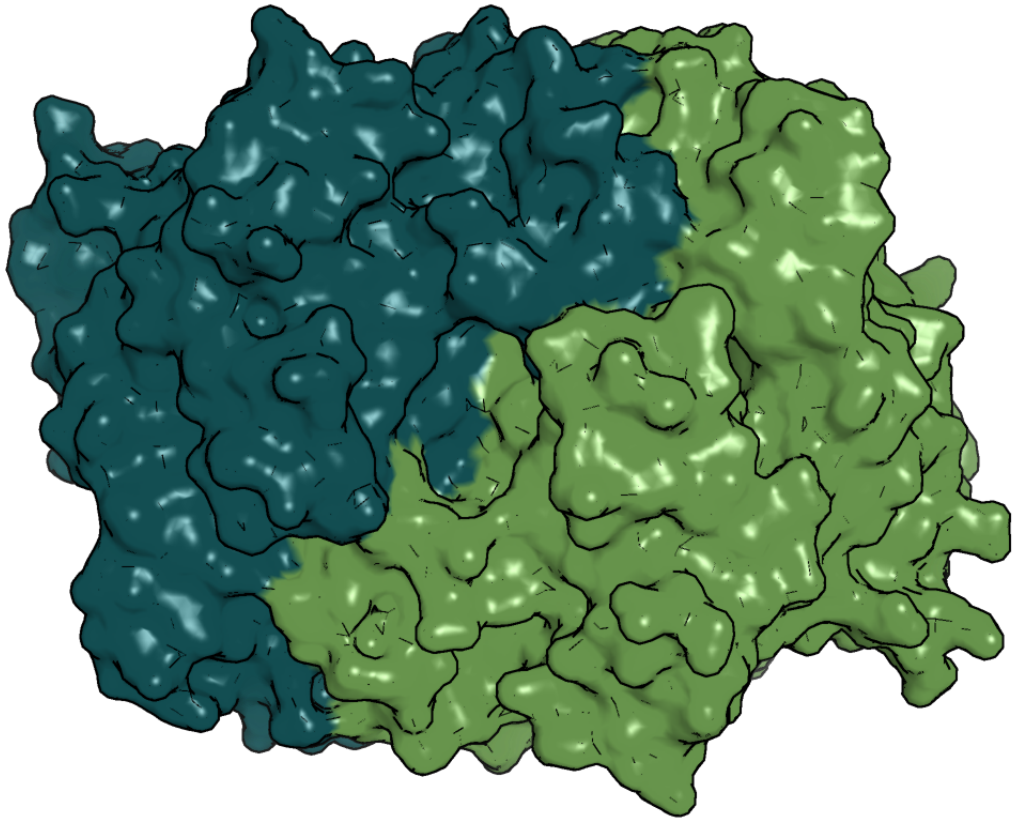


Chloroethane

Reductive Dehalogenases (RdhAs)



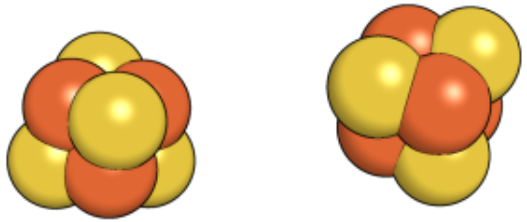
Cartoon representation



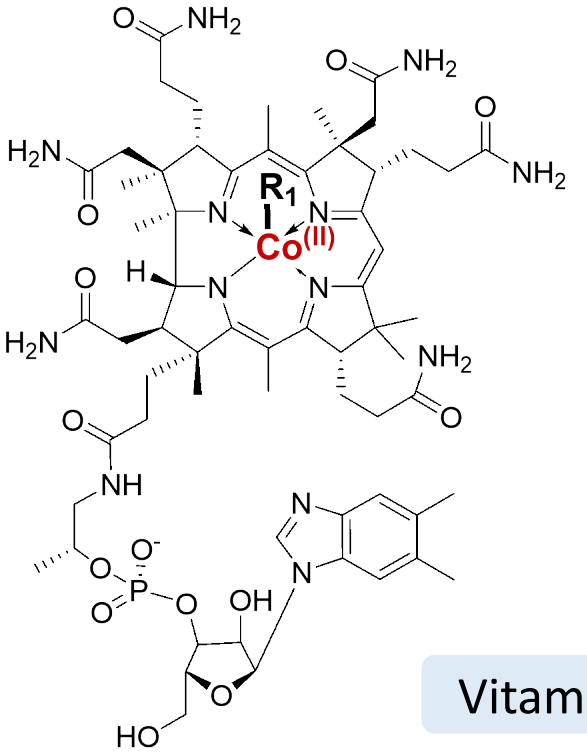
Surface representation

Structure PDB: 4UR0, Bommer, M. *et al. Science* (2014) **346:**455-458.

Reductive Dehalogenases (RdhAs)

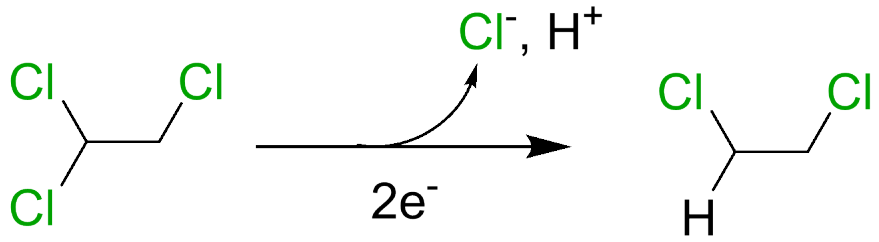


2x Iron-Sulfur clusters

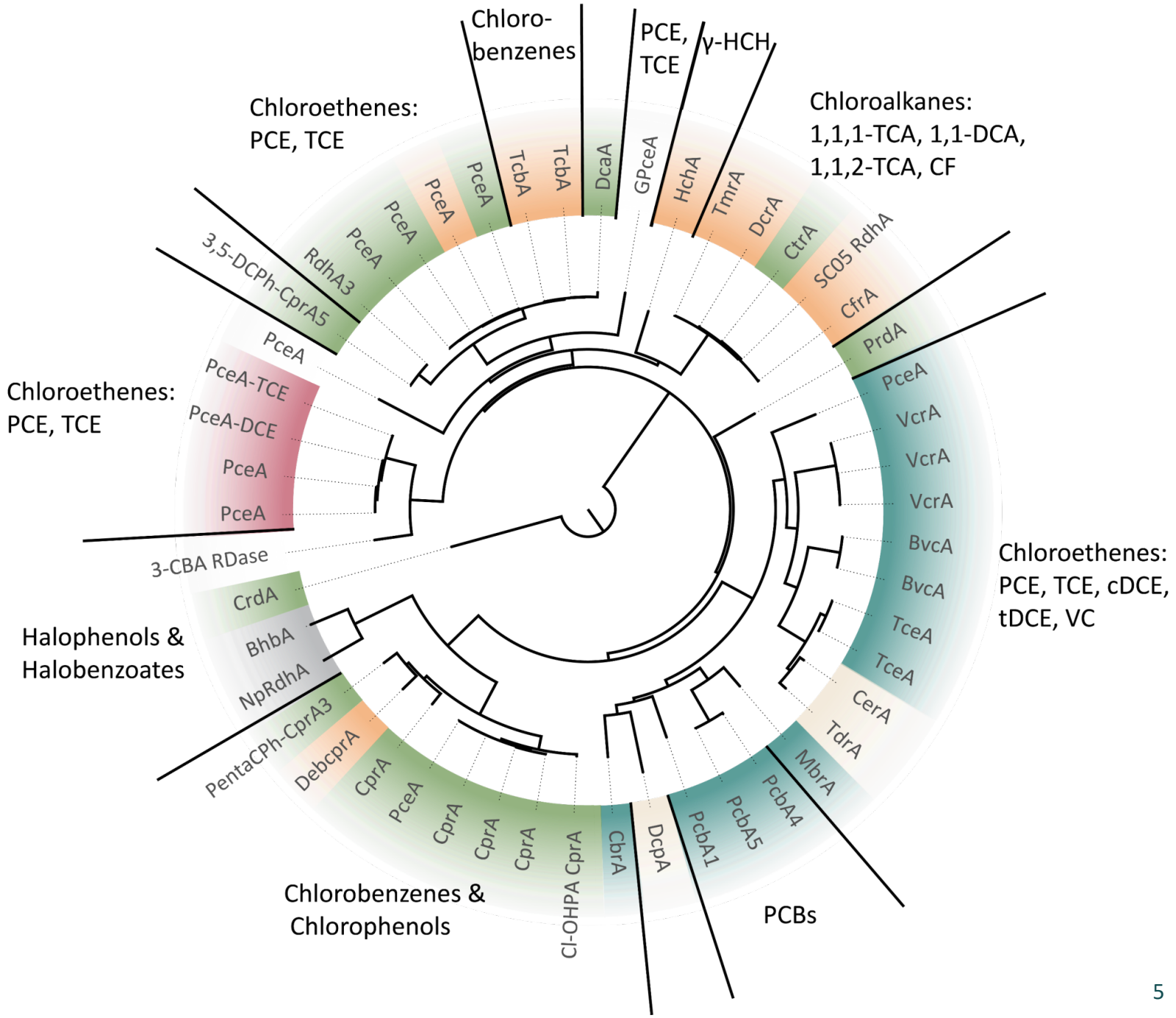


Vitamin B_{12}

Primary Mechanism



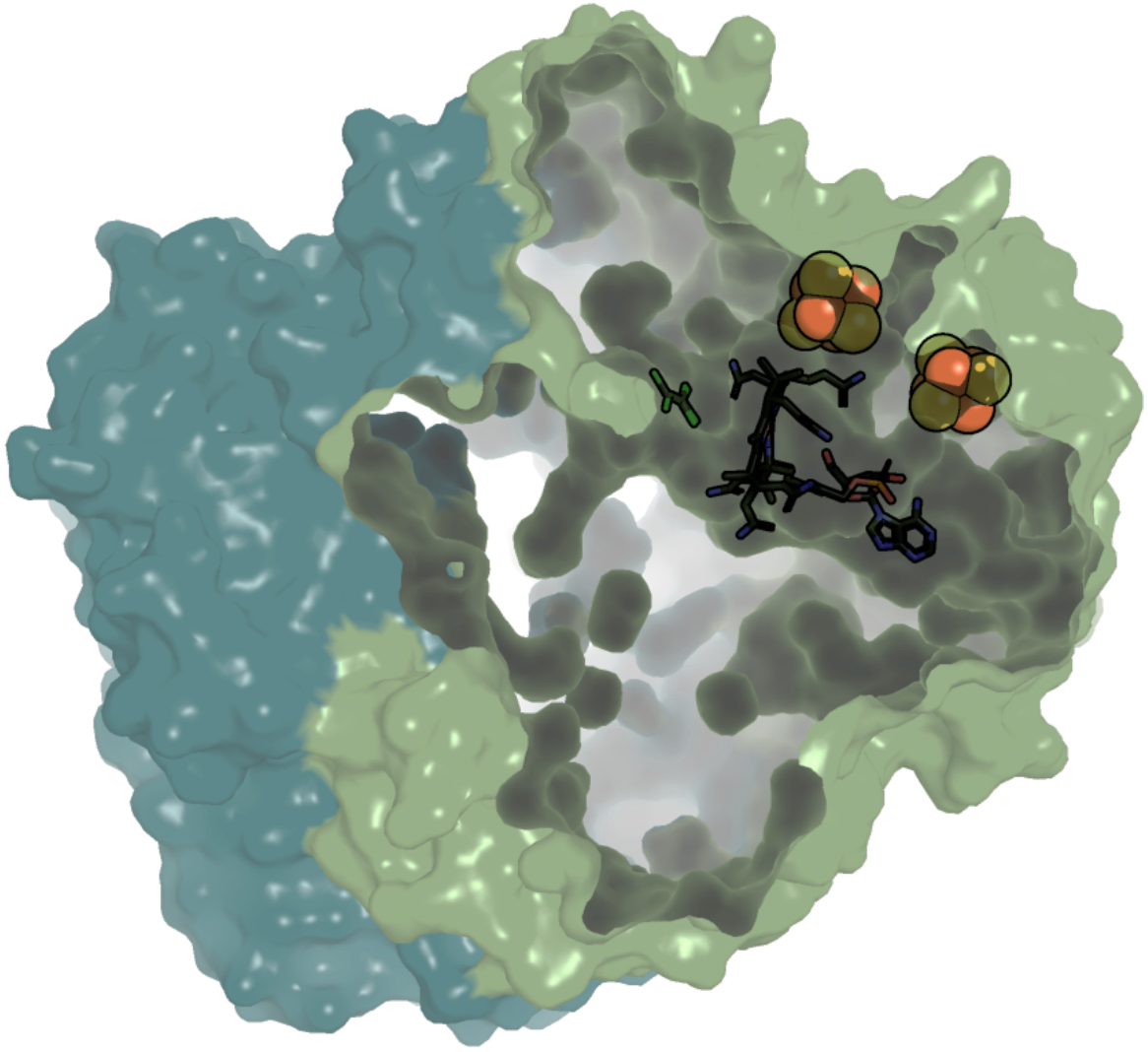
- > 1800 *rdhA* gene sequences in public metagenomic databases
- Only 25 unique RdhAs have experimentally determined function
- Characterization is usually done from small amounts of protein isolated from mixed culture



Dehalococcoides
Dehalogenimonas
Dehalobacter
Desulfitobacterium
Sulfurospirillum
Catabolic RDases
Other
0.6

Challenges with Heterologous Expression

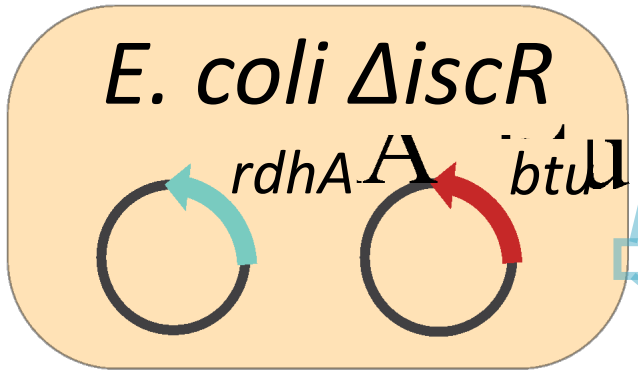
- Heterologous expression: using a host organism to produce a protein of interest
- B_{12} is deeply buried within enzyme and may be important in the structure
- *E. coli* does not synthesize B_{12}, and does not readily import it

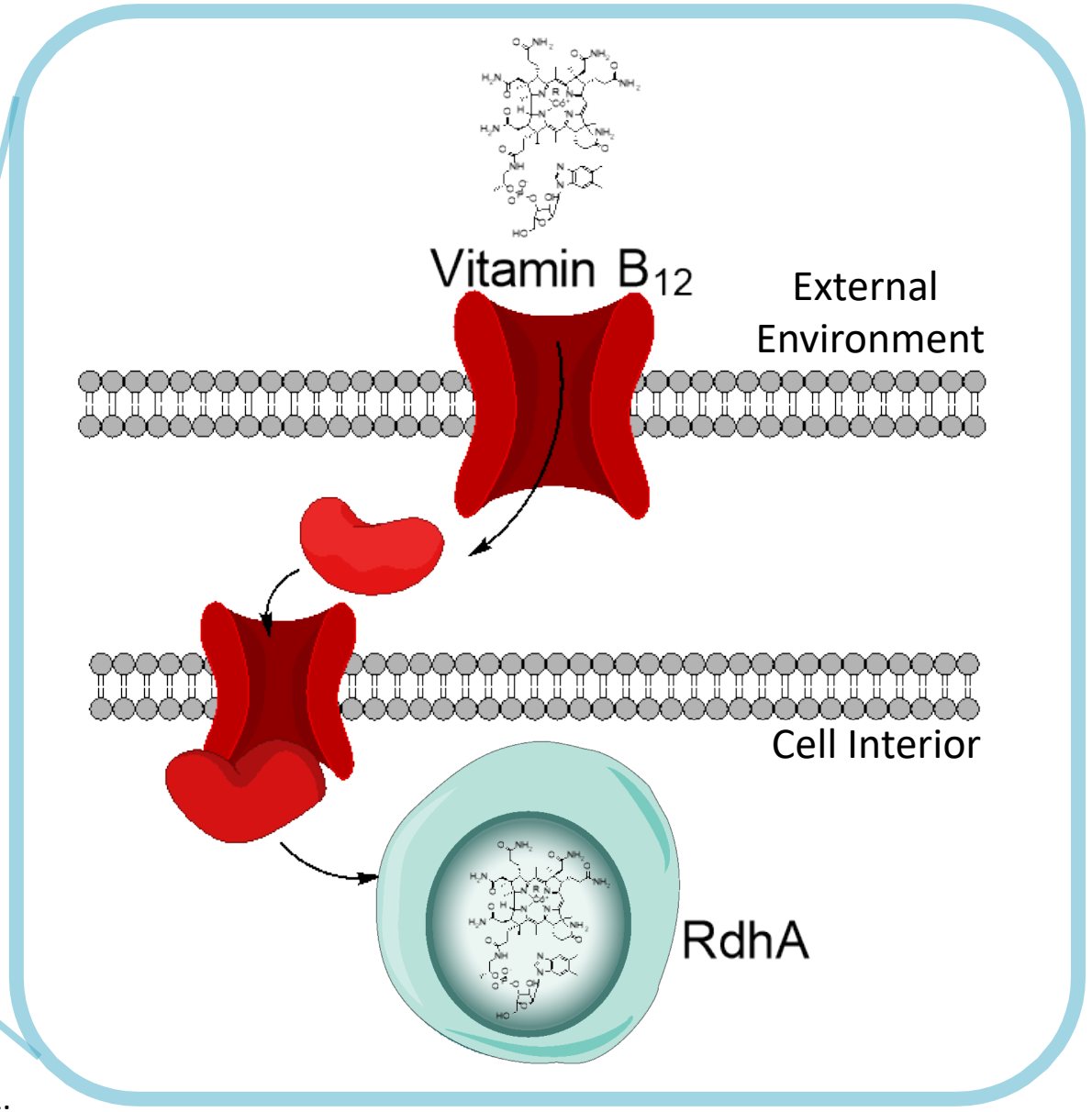


Structure PDB: 4UR0, Bommer, M. *et al. Science* (2014) **346:**455-458.

E. coli RdhA Expression System

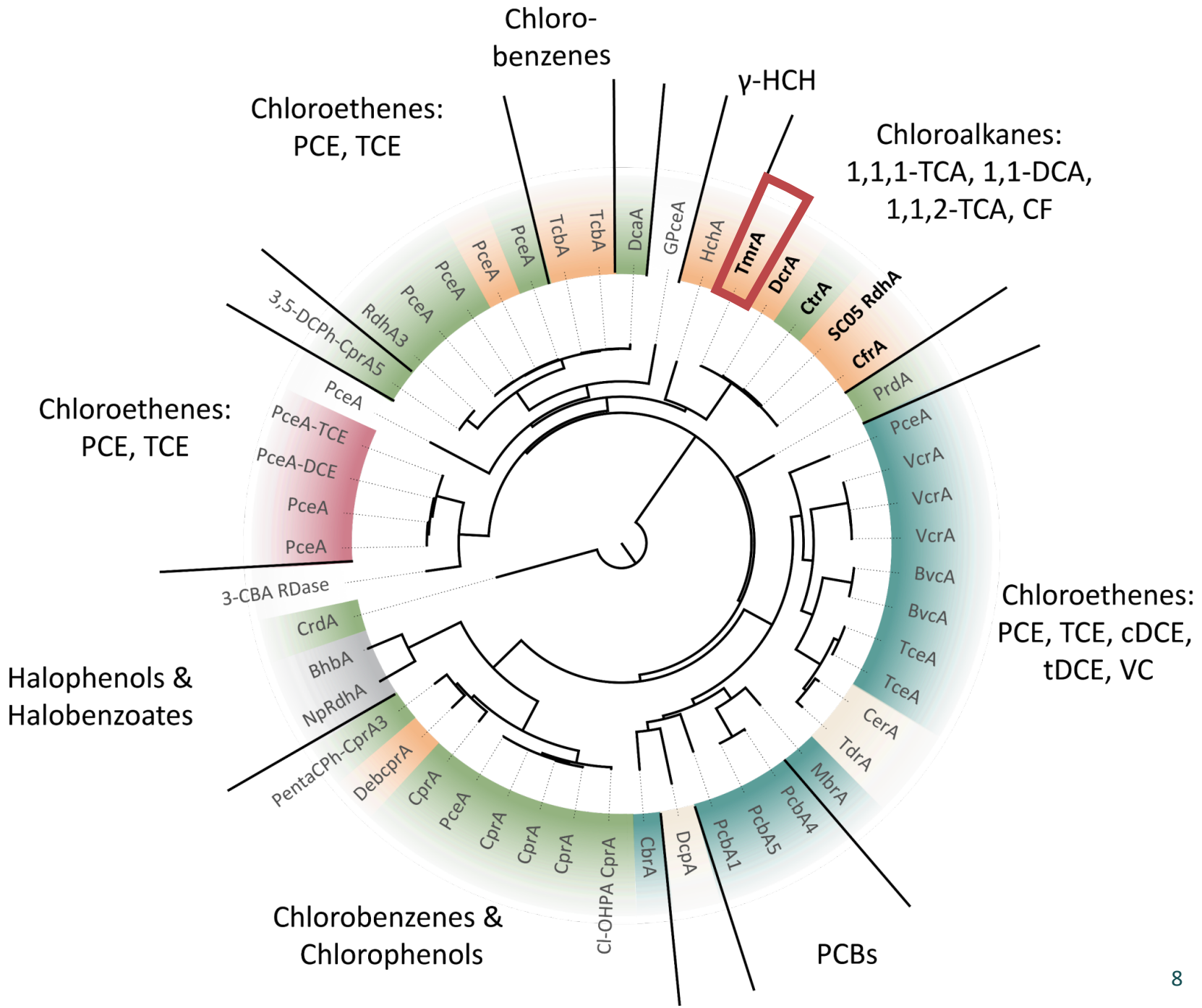
- Co-express vitamin B_{12} uptake (*btu*) pathway
- Use strain with enhanced Fe-S cluster production





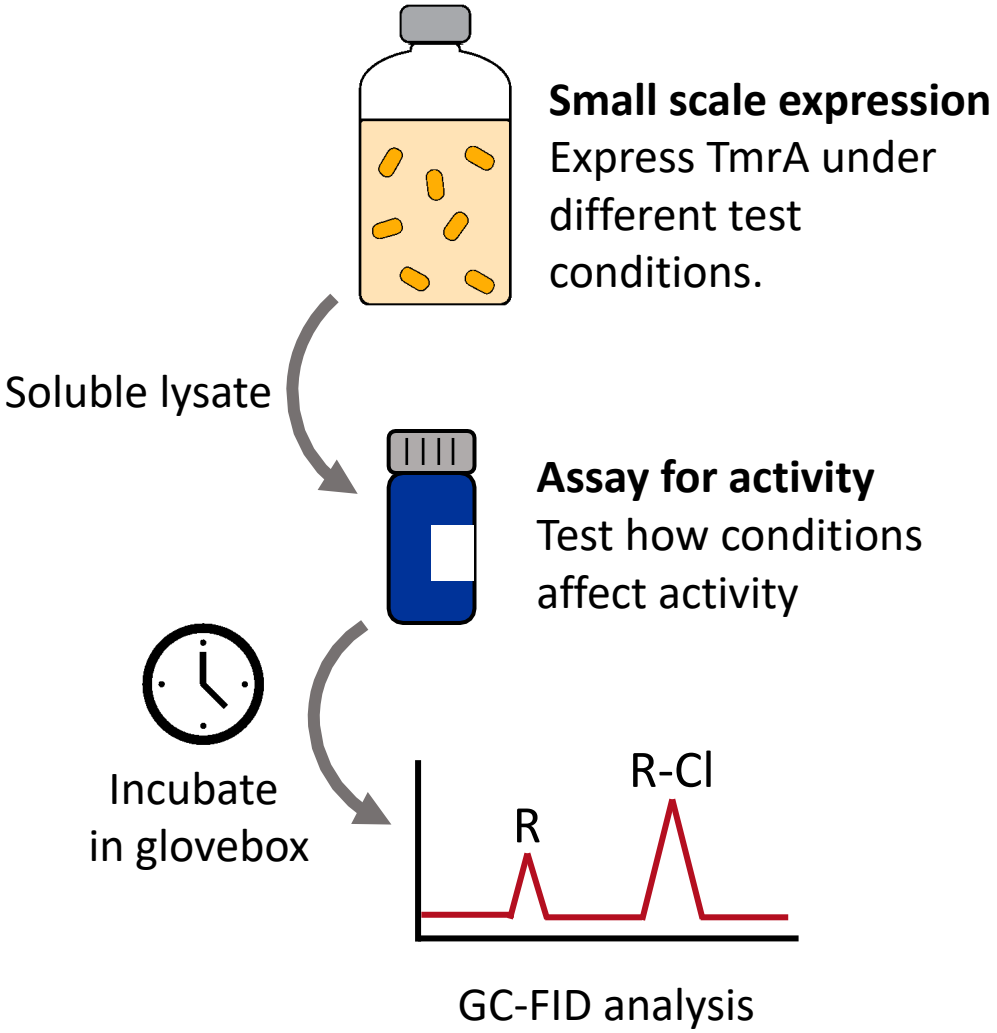
Picott, K.J. *et al. Appl. Enviro. Microbiol.* (2022) **88**: e01993-21.
Lanz, N. *et al. Biochemistry* (2018) **57:**1475-1490.
Akhtar, M. K. & Jones, P. R. *Appl. Microbiol. Biotechnol.* (2008) **78**:853-862.

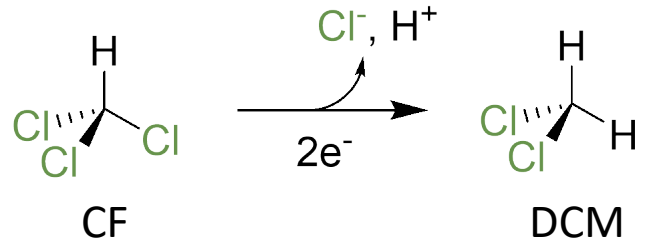
- TmrA from *Dehalobacter* sp. UNSWDHB
- Primary substrates are chloroform and 1,1,1-trichloroethane

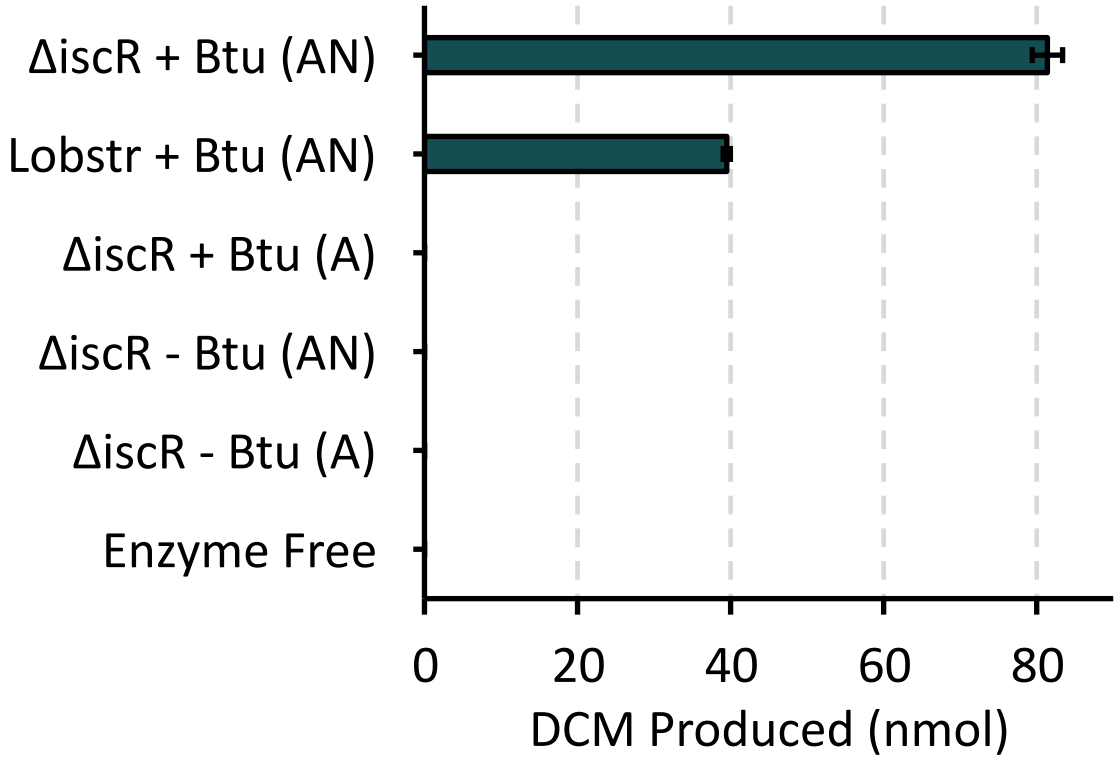


Jugder B. *et al. ACS. Chem. Biol.* (2018) **13**:548-552.

Optimizing TmrA Expression Conditions





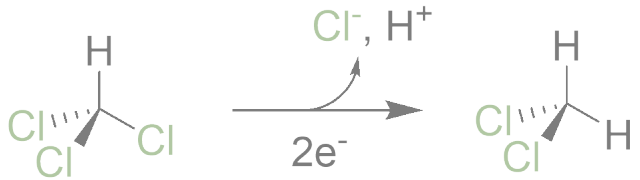


With (+)/without (-) Btu; A = aerobic; AN = anaerobic, ΔiscR, Lobstr = *E. coli* strains

Picott, K.J. *et al. Appl. Enviro. Microbiol.* (2022) **88**: e01993-21.

Optimizing TmrA Expression Conditions

Small scale expression
Express TmrA under

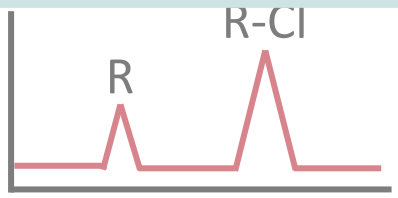


CF DCM

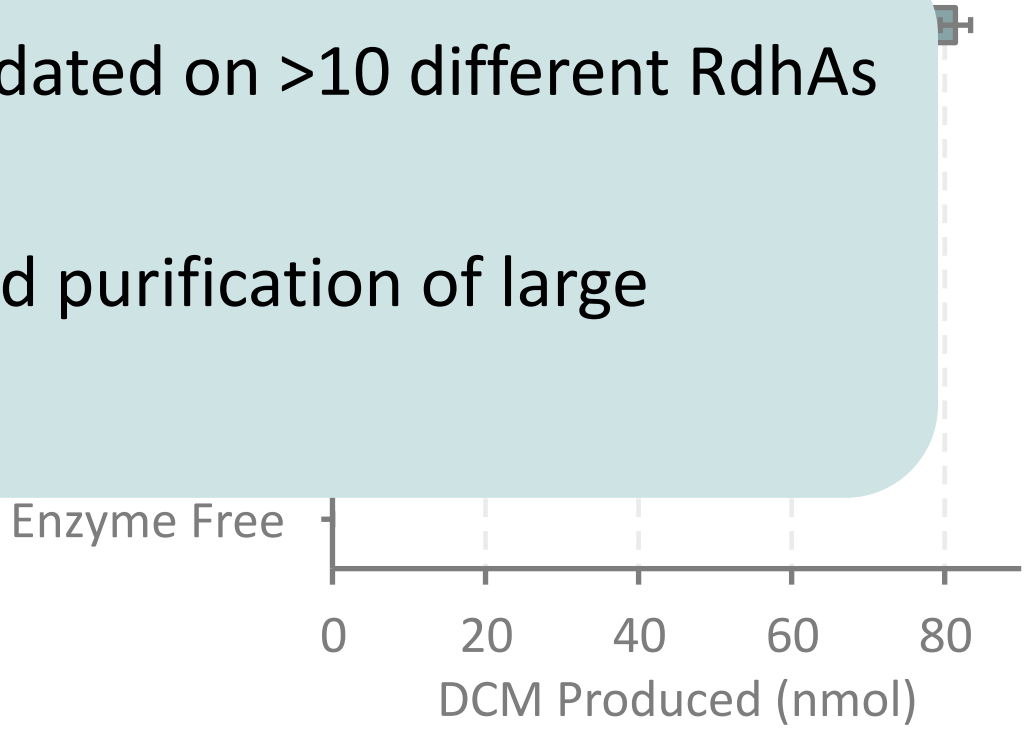
- Expression system has been validated on >10 different RdhAs from *Dehalobacter* sp.
- System allows for production and purification of large amounts of enzyme

Soluble lysa

Incubate
in glovebox



GC-FID analysis



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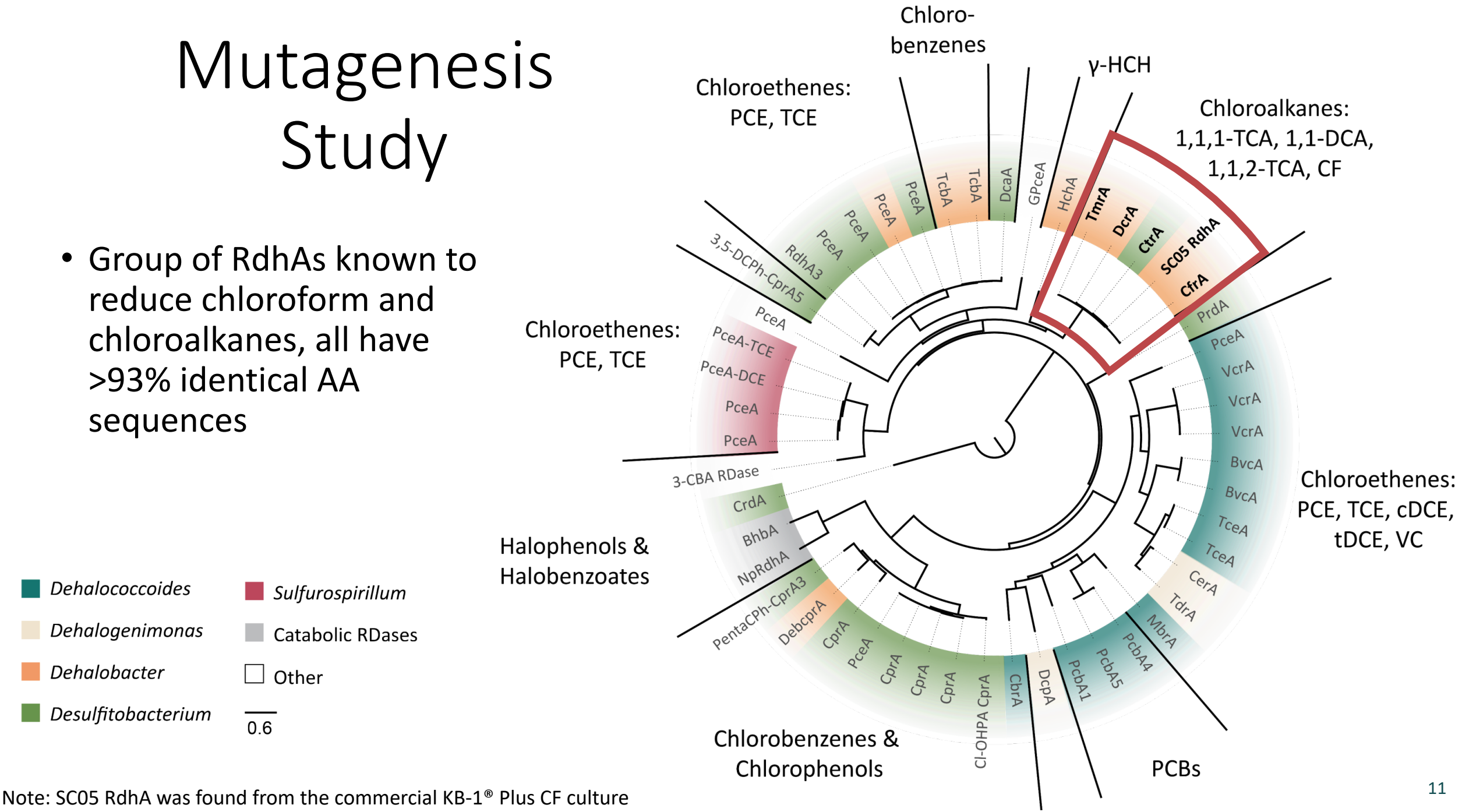
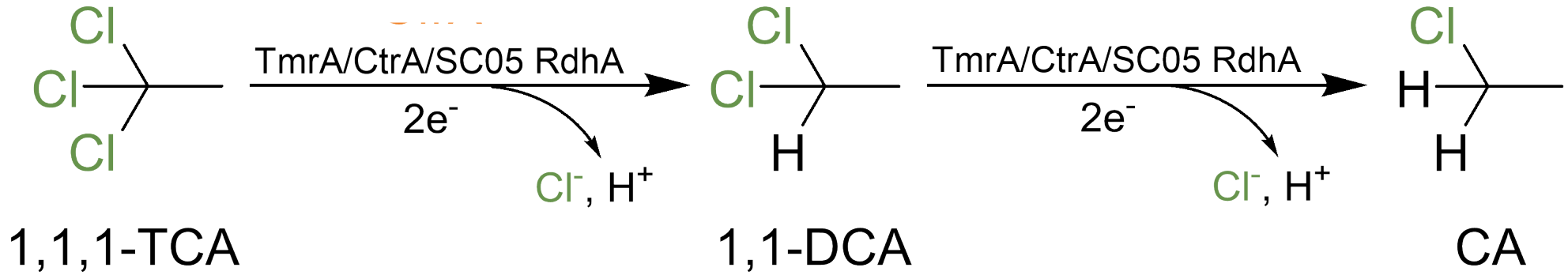
Mutagenesis Study

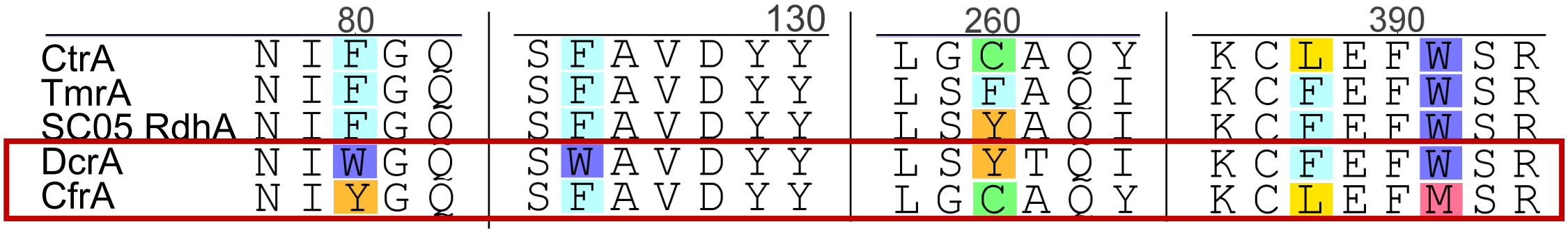
- Group of RdhAs known to reduce chloroform and chloroalkanes, all have >93% identical AA sequences



Note: SC05 RdhA was found from the commercial KB-1® Plus CF culture

Chloroethane Reductases



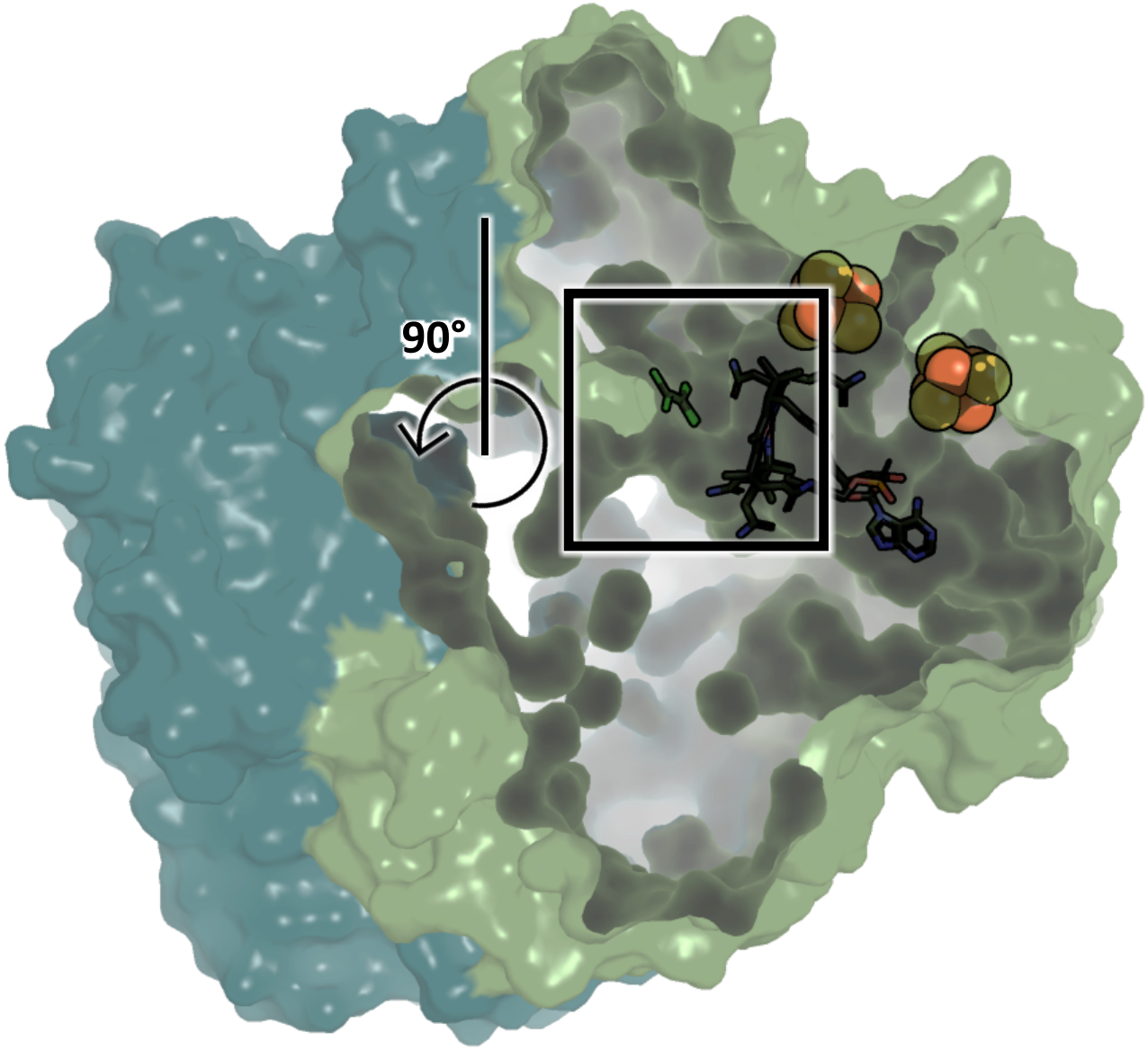


Hydrophobic Aromatic (ring structures)
Phenylalanine (F), Tryptophan (W), Tyrosine (Y)

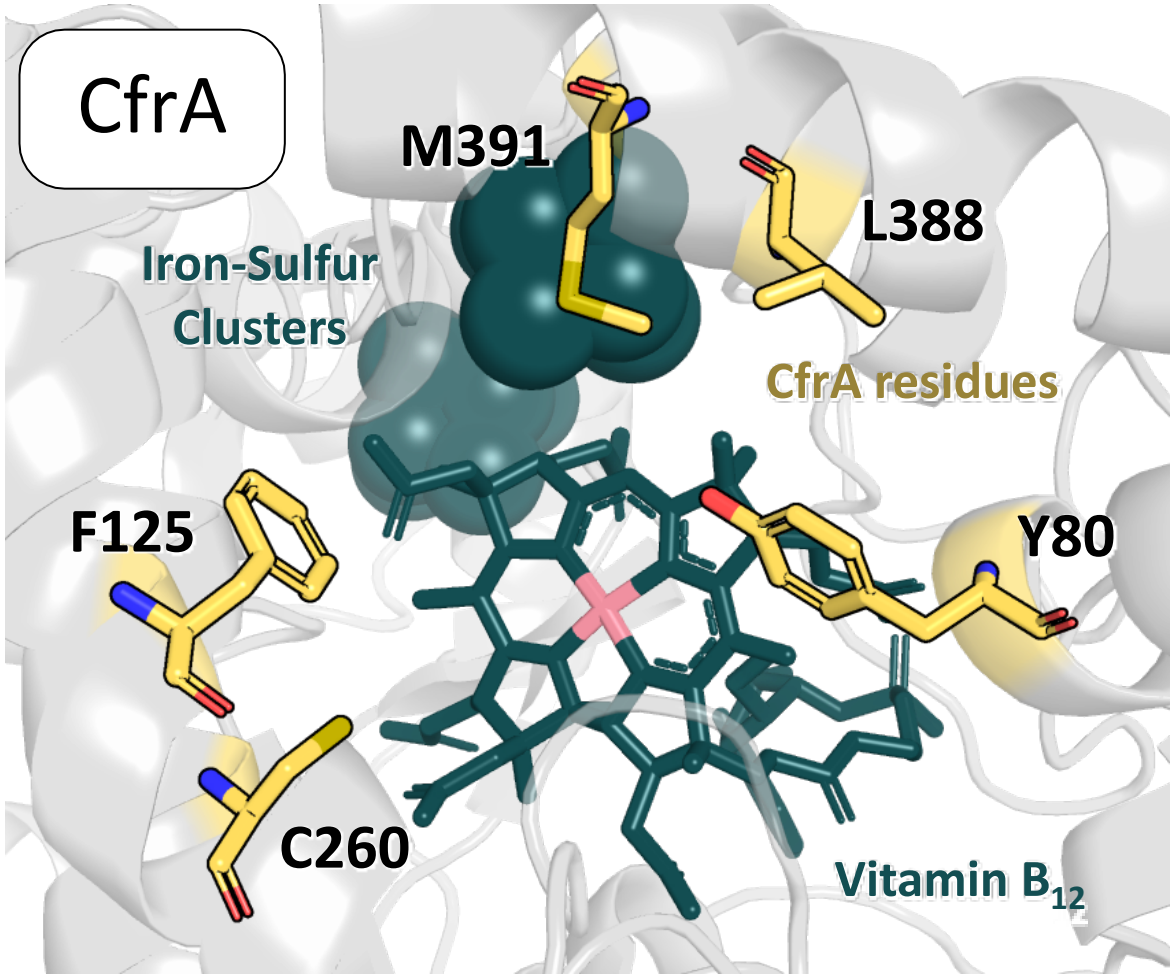
Hydrophobic Aliphatic (linear carbon chains)
Cysteine (C), Leucine (L), Methionine (M)

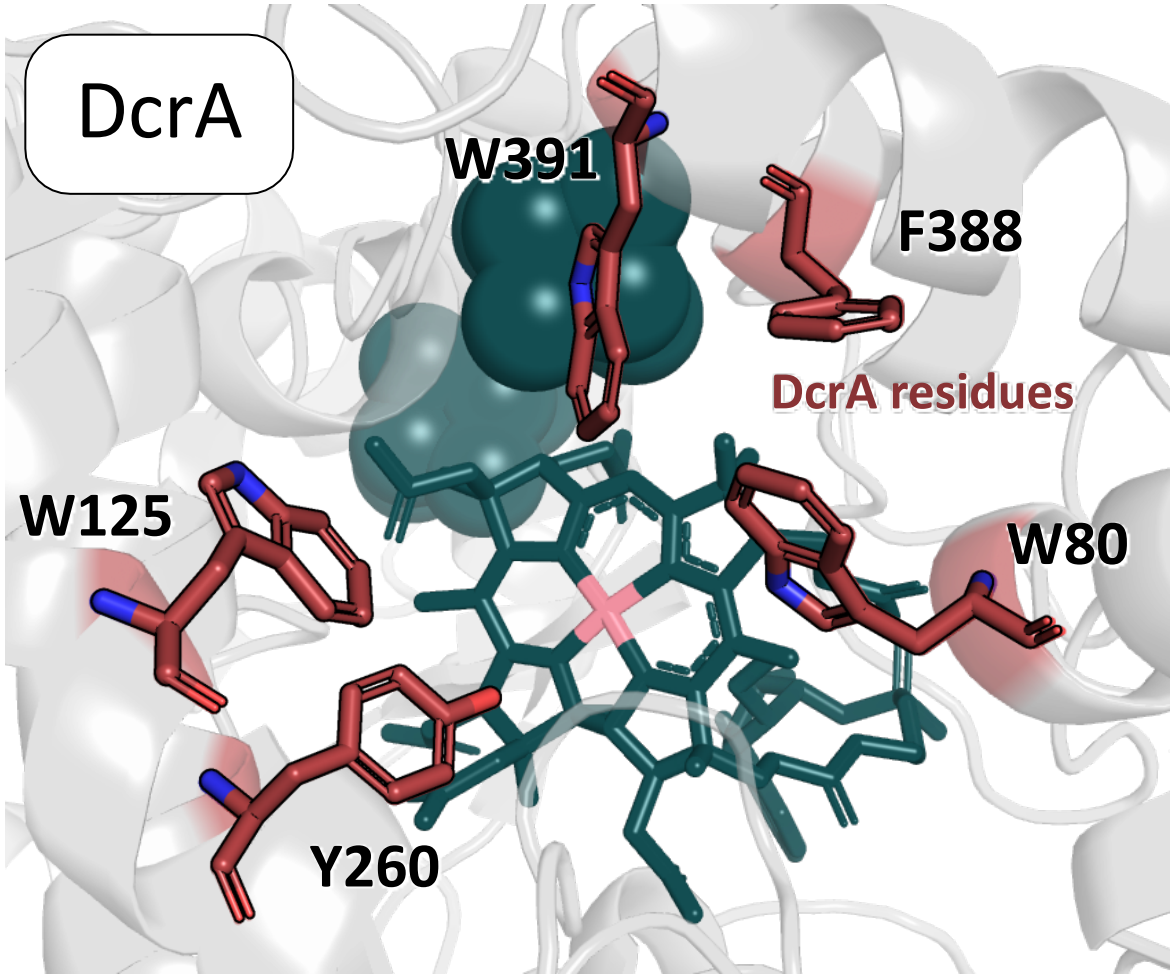
Phillips. E. *et al. FEMS Microbiol. Ecol.* (2022) **98:**fiac072.

Active Site Architecture



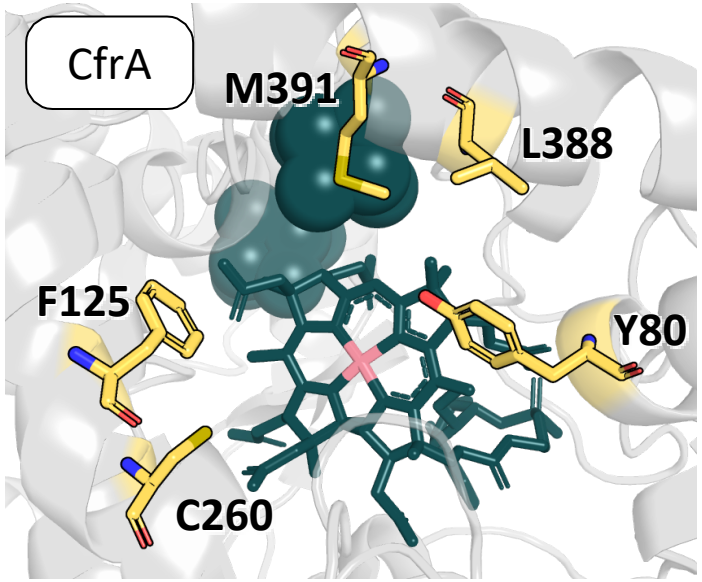
Active Site Architecture

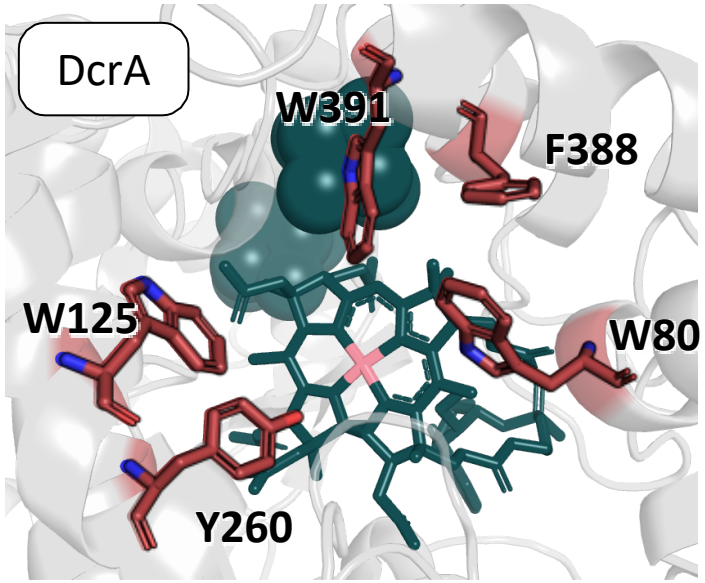


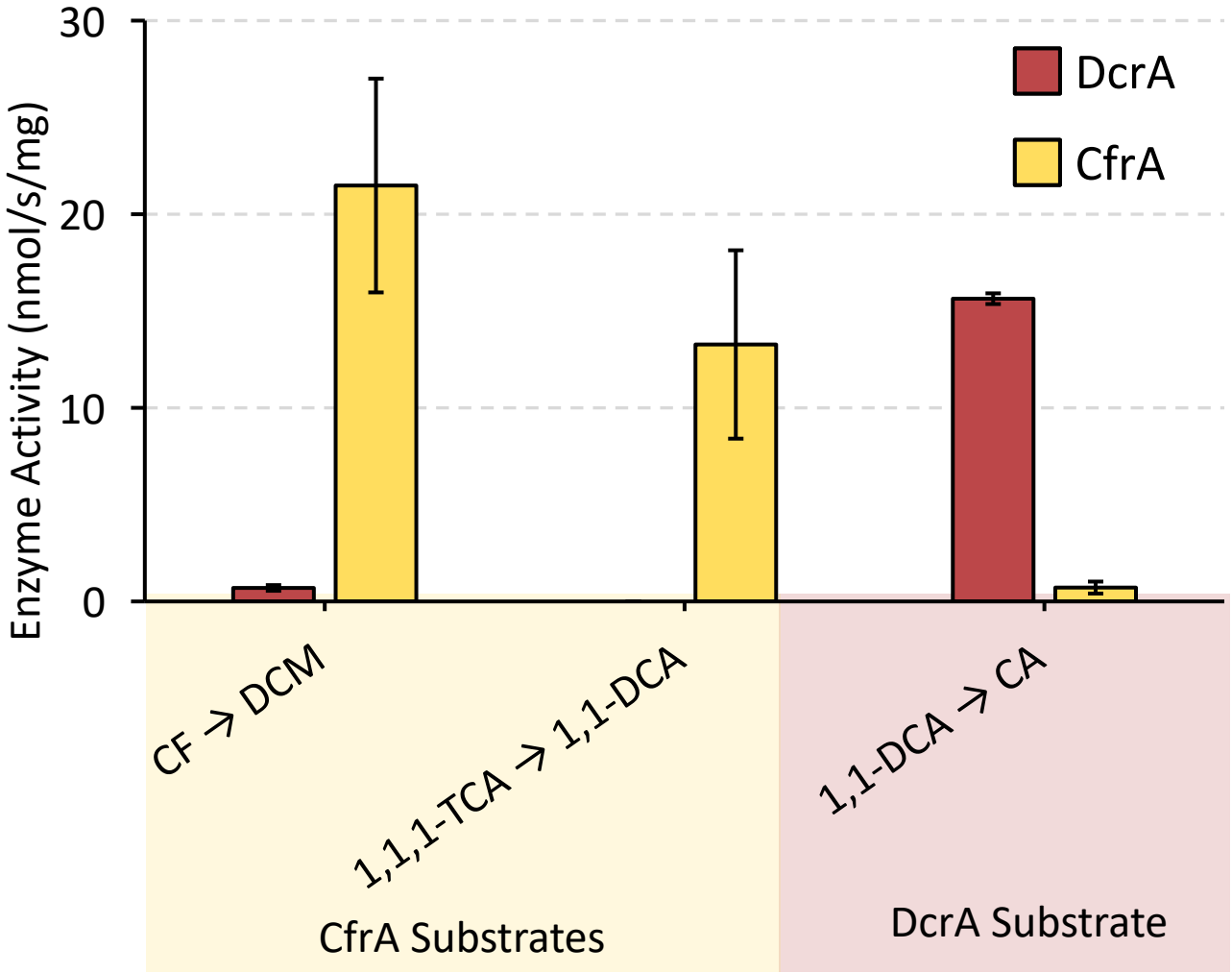


[AA][Residue Number]

Active Site Architecture

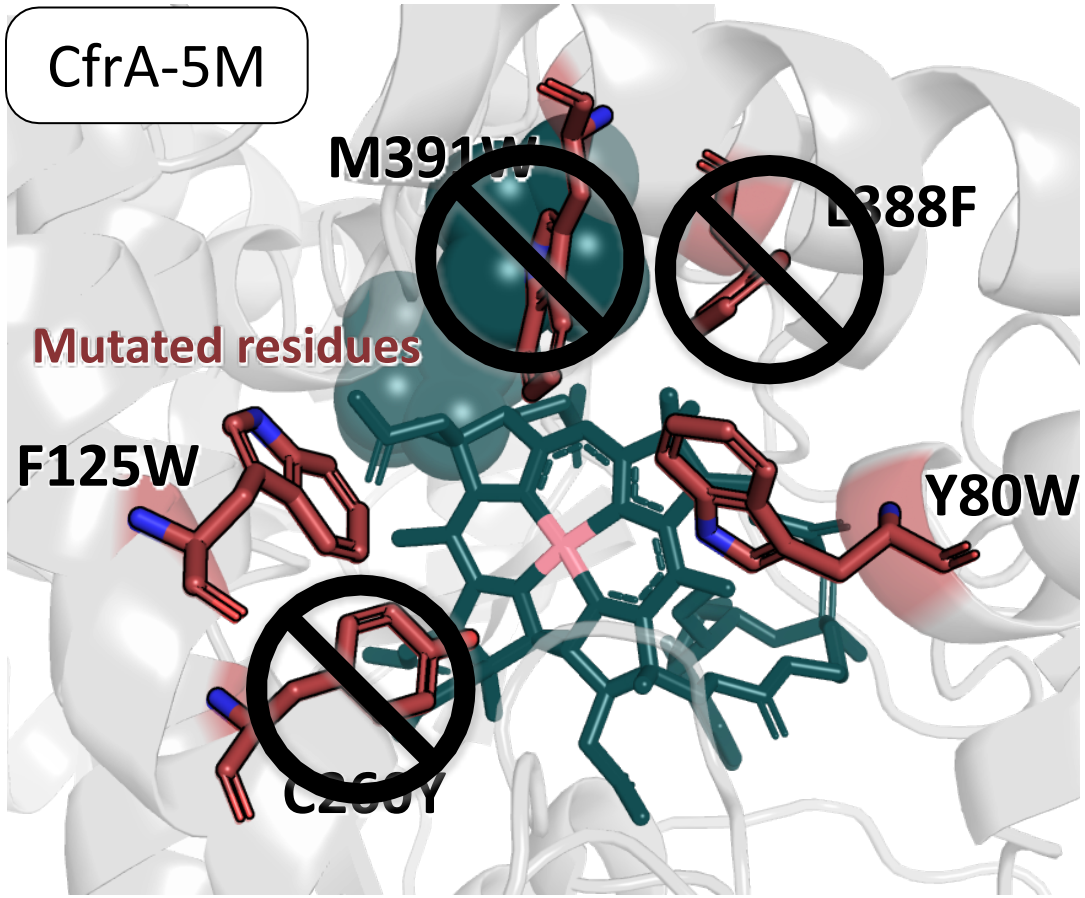






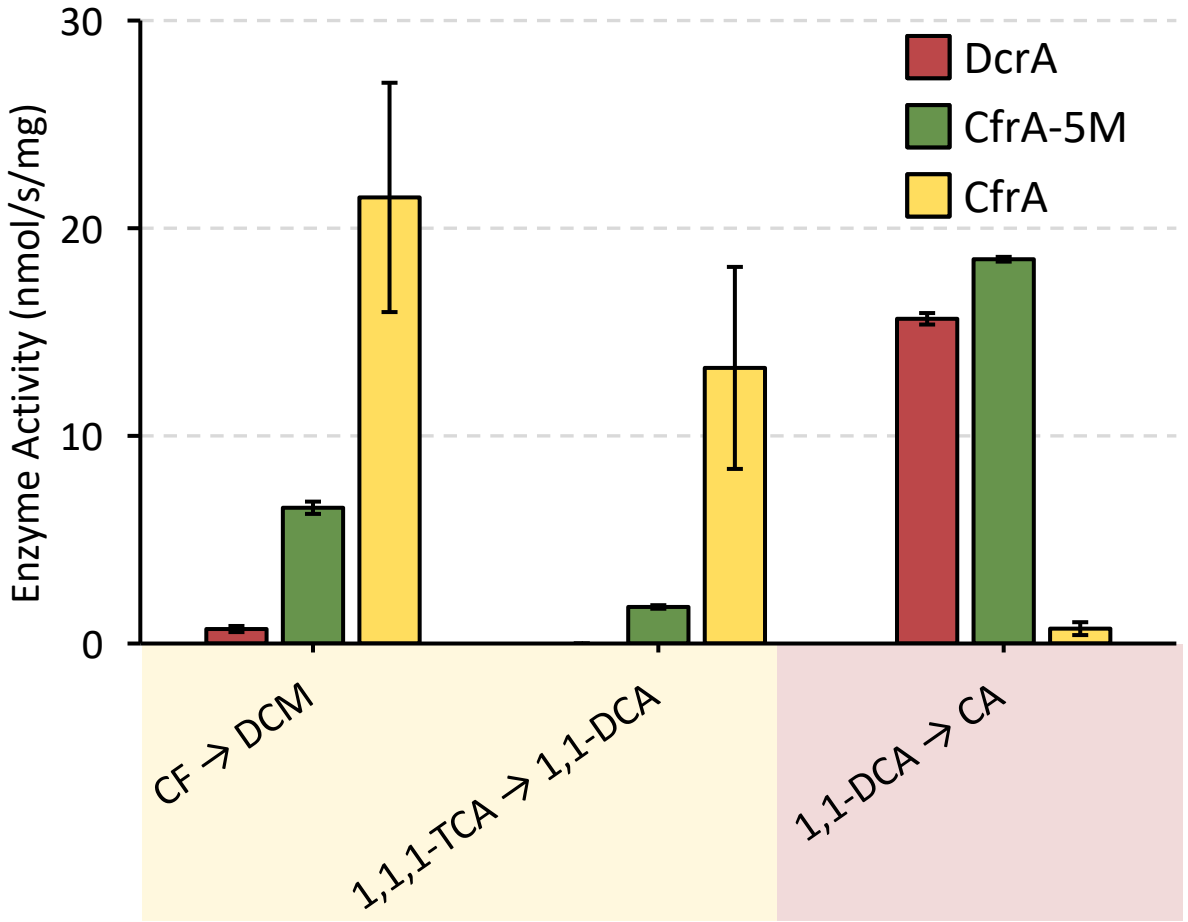
CF = chloroform; DCM = dichloromethane; TCA = trichloroethane; DCA = dichloroethane; CA = chloroethane

CfrA – 5x Mutant



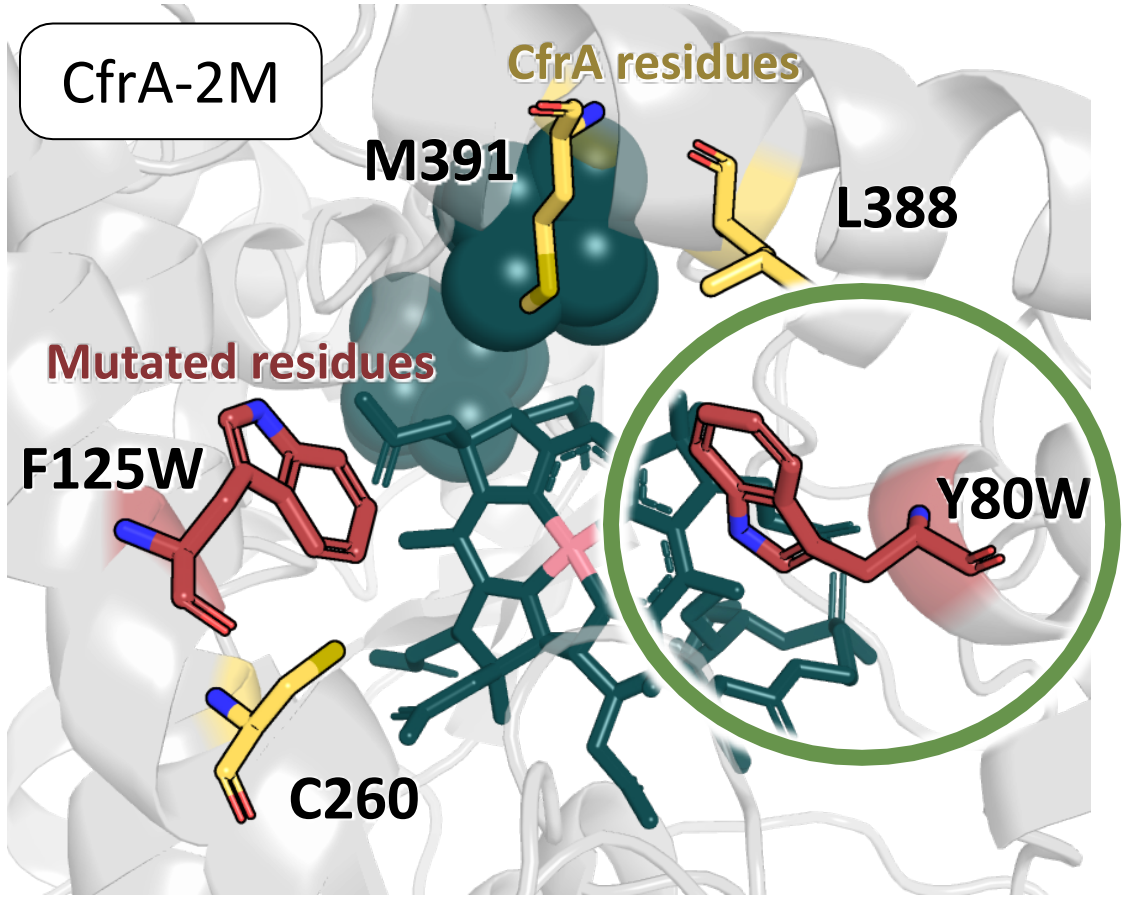
[Original AA][Residue][Mutant AA]

Cofactors: B_{12}, iron-sulfur clusters



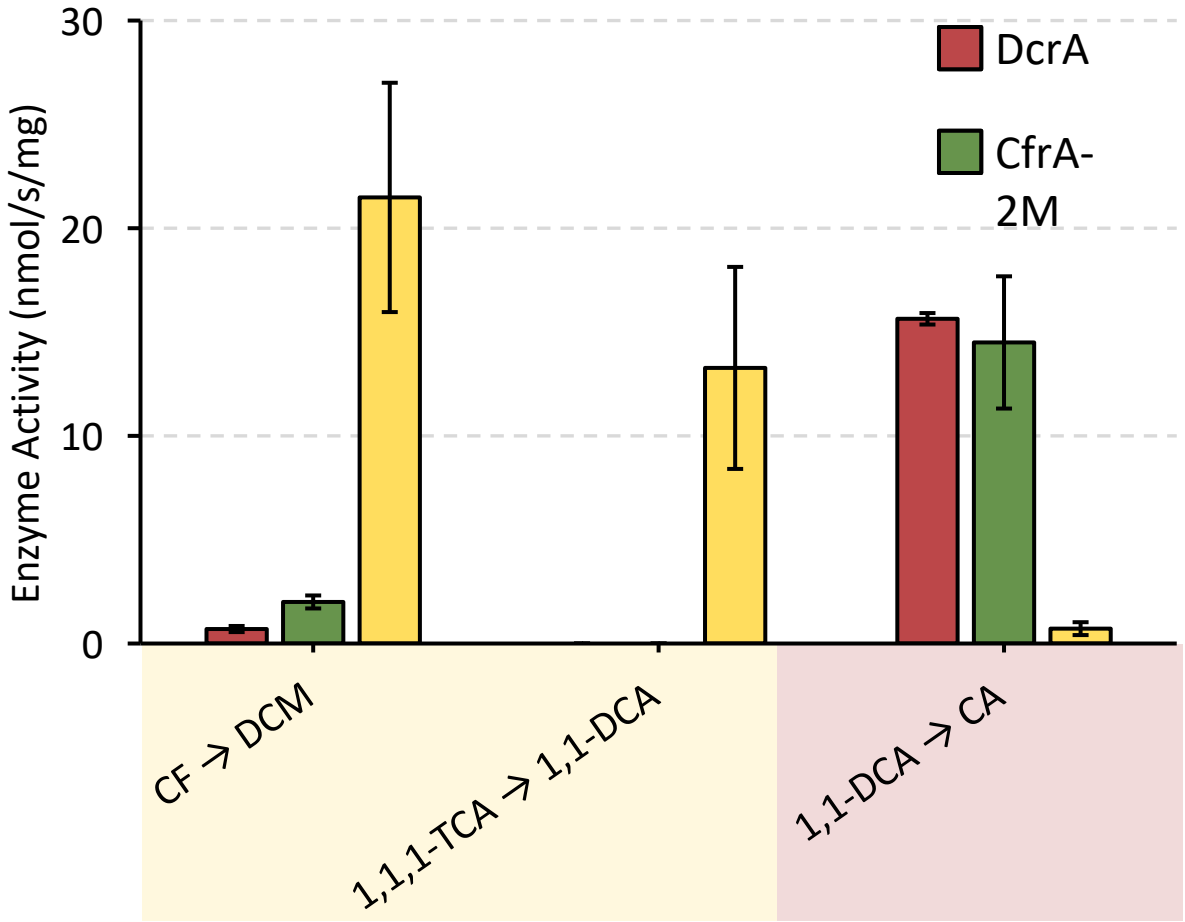
CF = chloroform; DCM = dichloromethane; TCA = trichloroethane; DCA = dichloroethane; CA = chloroethane

CfrA – Double Mutant



[Original AA][Residue][Mutant AA]

Cofactors: B_{12}, iron-sulfur clusters



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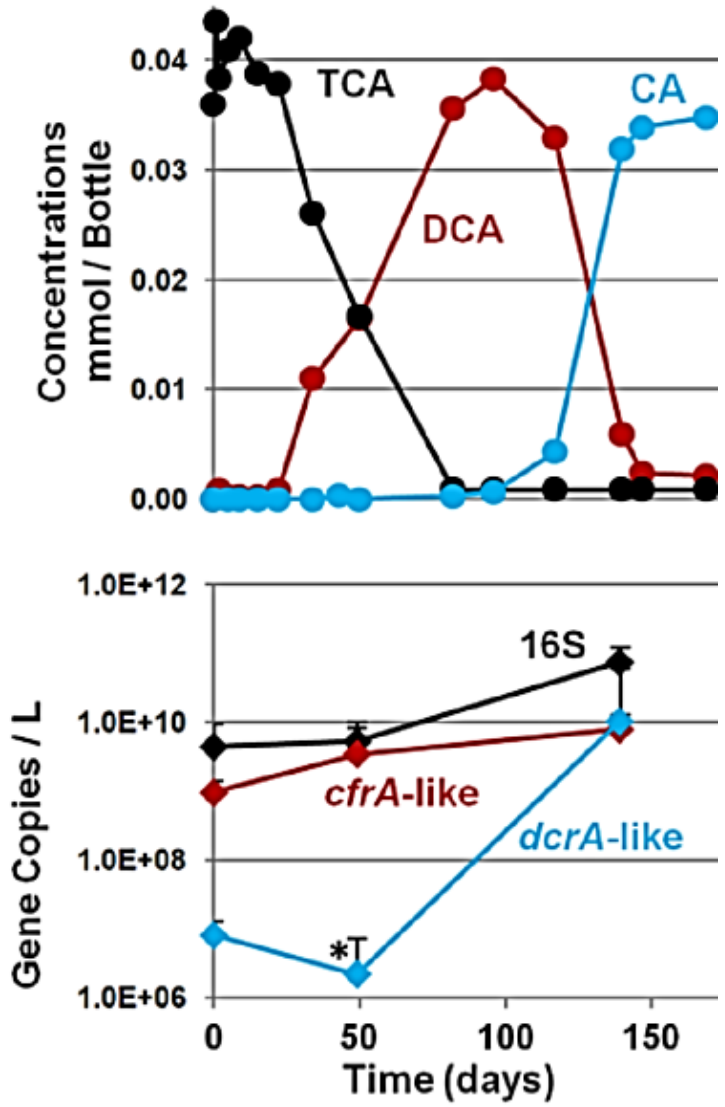
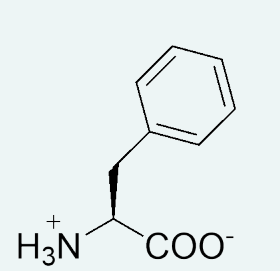
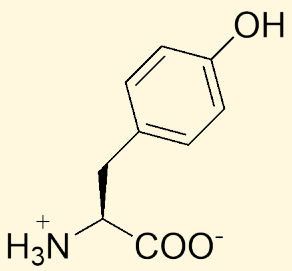
Applied to *rdhA* Sequences from Field



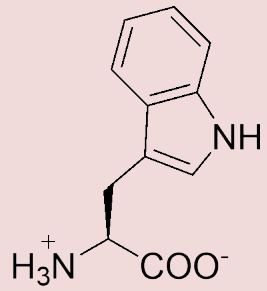
	Residue position				
	80	125	260	388	391
CfrA	Y	F	C	L	M
CfrA-like clone 1	F	F	C	L	W
CfrA-like clone 2	Y	F	F	L	F
DcrA	W	W	Y	F	W
DcrA-like	W	W	Y	F	W



Phenylalanine (F)



Tyrosine (Y)

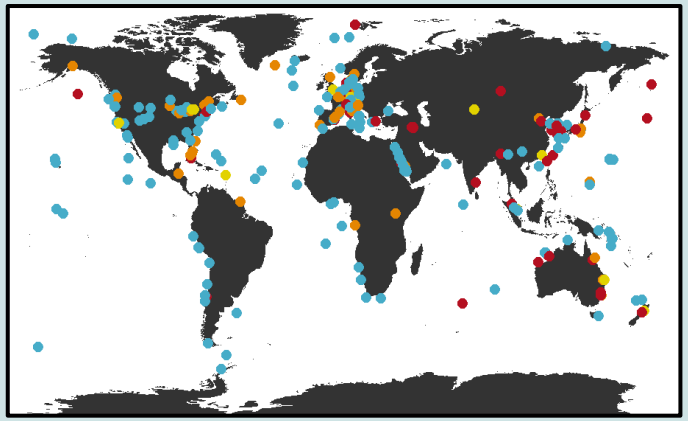


Tryptophan (W)

Yang, M.I. *et al. Water Res.* (2020) **186:**116310.

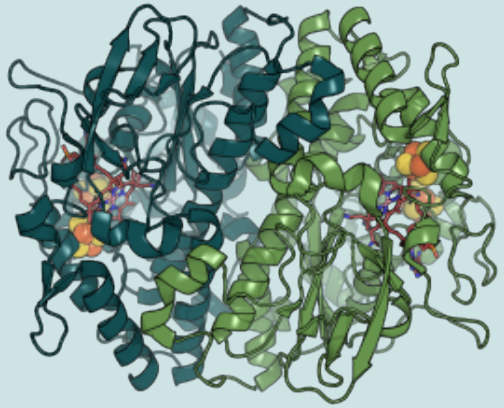
Other Applications

RdhA Discovery

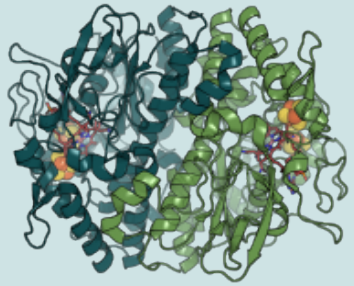


Biochemical studies

- X-ray crystallography
- Substrate binding
- Enzyme kinetics



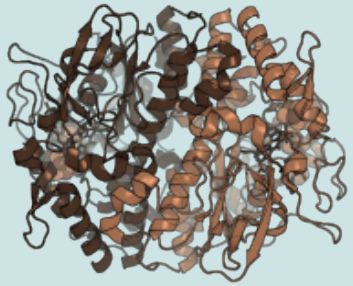
Enzyme Engineering



$Cl\text{-}R_{Native}$

R_{Native}

Mutation



$Cl\text{-}R_{New}$

R_{New}

Significance and Conclusions

- First reliable method to express RdhAs in *E. coli*
- Reproducible system for obtaining large amounts of RdhAs for direct interrogation of their activity
- System allows for modification of the enzymes to obtain new activity

Acknowledgements

Edwards Lab

- Dr. Elizabeth Edwards
- Connor Bowers, Liam Foyle, Line Lomheim, Olivia Bulka, Endang Susilawati, Vinthiya Paramananthasivam

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- Dr. Sofia Lemak
- Dr. Krishna Mahadevan

BioZone
Centre for Applied Bioscience and Bioengineering

NSERC
CRSNG

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Thank you for your attention!

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