Structural basis of the enantioselectivity of a pathogenic epoxide hydrolase

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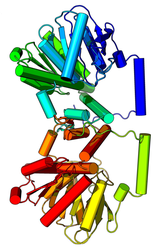
1. Background – i. aCif is an α/β hydrolase[1]

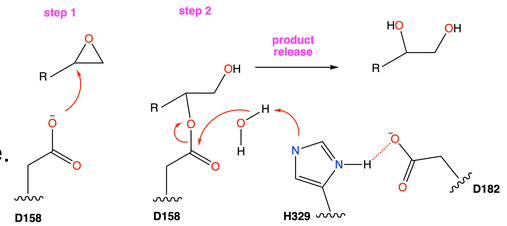
- aCif (*Acinetobacter* CFTR inhibitory factor) is a virulence factor with epoxide hydrolase activity.
- aCif is a member of the α/β hydrolase super family, consisting of a core domain and a lid domain.
- aCif has a N-terminal extension distinct from its homolog Cif (from *P*. aeruginosa).
- aCif is a dimer. Within each monomer, the active site is on the interface between the core and lid domain.



aCif dimer

ii. 2-step hydrolysis of styrene oxide by aCif[1,3-4]

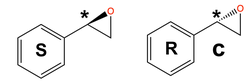
- The epoxide oxygen binds to the His-Tyr oxyanion hole.
- Nucleophilic attack by D158 opens the epoxide ring and forms an adduct intermediate.
- Charge-relay D182-H329 activated water attacks the adduct and releases product.



Scheme 1. catalytic cycle of aCif. Adapted from Bahl, C.D., *et al.* (2014) *J Biol Chem* 289: 7460-7469

iii. Enantioselectivity of aCif[1]

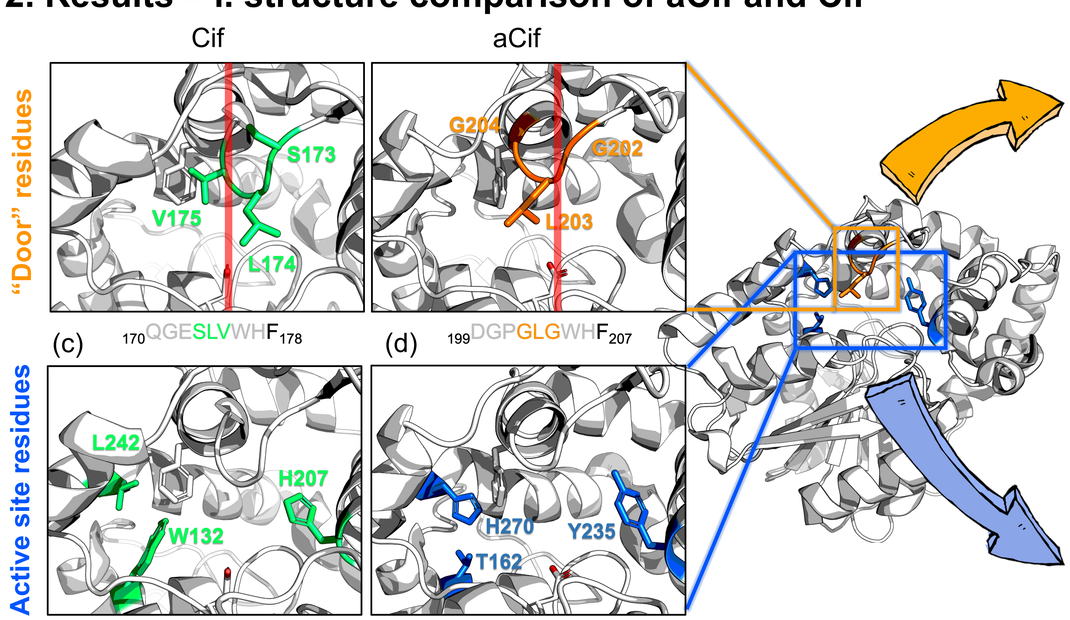
- aCif shows distinct enantioselectivity for the model substrate Styrene Oxide (SO) compared to Cif.
- aCif only hydrolyses S-SO, while Cif shows preference for R-SO over S-SO.



*chiral carbon

	Activity	
	S-SO	R-SO
Cif	+ +	+ + +
aCif	+ +	-

2. Results – i. structure comparison of aCif and Cif

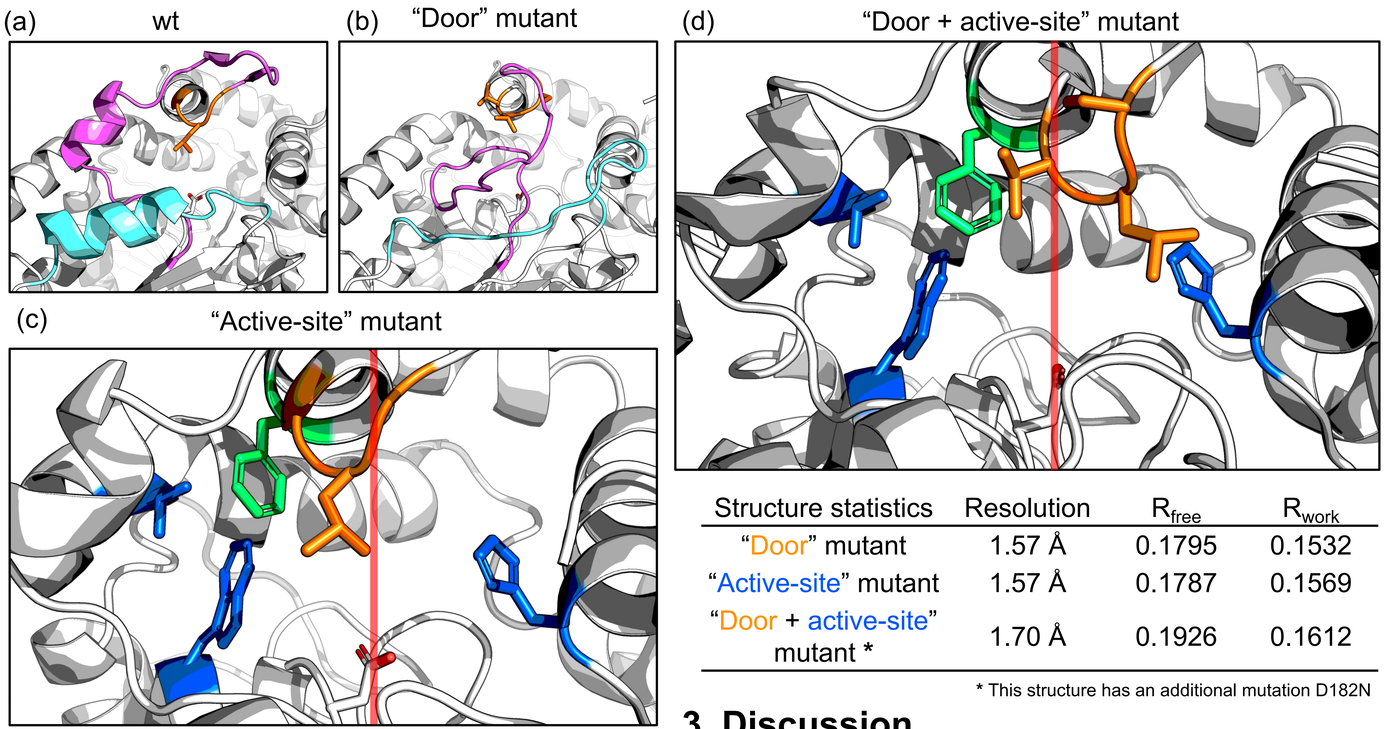


- To investigate the structural basis of the enantioselectivity of aCif, the sequences and structures of aCif [1] (PDB ID: 4MEA) and Cif [2] (PDB ID: 3KD2) are analyzed.
- Both aCif and Cif have leucine "door" residues at the substrate tunnel. However, their sequences and structures differ. The compositions of active-site residues also differ but their main-chain conformations are conserved.

ii. Mutation designs and activity assay

	Mutations					Activity	
wt	T162	Y235	G202	G204	Y270	S-SO	R-SO
"Door" mutant	-	-	S	V	-	-	-
"Active-site" mutant	W	H	-	-	H	+ +	-
"Door + active-site" mutant	W	H	S	V	H	+ +	+

iii. Crystal structures of aCif mutants



(Solid red line indicates the relative position of L203 to nucleophilic D158)

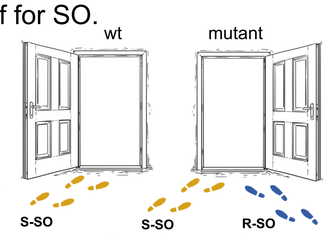
- The "door" mutations alone (b) rearrange the lid domain (pink) and unfold an adjacent helix (cyan).
- "Active-site" mutation's side-chain conformation (c) is closer to Cif than aCif. Interestingly, F207 (green) employs another rotamer similar to Cif. The "door" residues are unchanged.
- When 'door' mutations are combined with "active-site" mutations (d), the conformation of "door" residues are similar to those in Cif.

Structure statistics	Resolution	R_{free}	R_{work}
"Door" mutant	1.57 Å	0.1795	0.1532
"Active-site" mutant	1.57 Å	0.1787	0.1569
"Door + active-site" mutant *	1.70 Å	0.1926	0.1612

* This structure has an additional mutation D182N

3. Discussion

- Mutagenesis, activity assays, and X-ray crystallography indicate that the conformation of "door" residues is crucial for the enantioselectivity of aCif for SO.
- For L203 to employ a Cif-like conformation, structural "support" is required from active site resides, indicating the co-evolution of structurally-related sites.



* Image credit – adobe.com, adapted

Reference

1. Bahl, C.D., *et al*. (2014) *J Biol Chem* 289: 7460-7469
2. Bahl, C.D., *et al*. (2010) *J Bacteriol* 192: 1785-1795
3. Bahl, C.D., *et al*. (2016) *Biochemistry* 55: 788-797
4. Hvorecny, K.L., *et al*. (2017) *Structure* 25, 697–707

Acknowledgements

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