Allosteric and Cooperative Networks in a Homodimeric Epoxide Hydrolase

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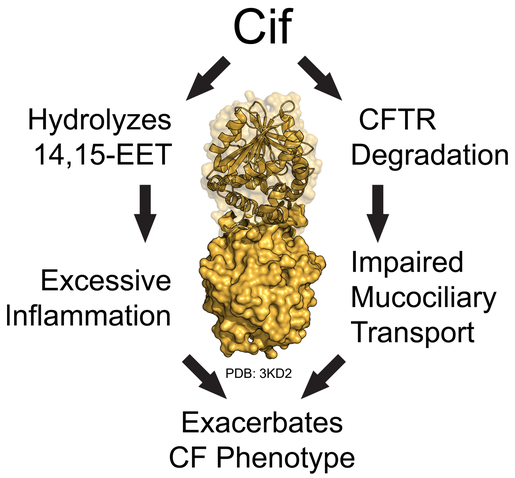
MCB The Molecular and Cellular Biology Graduate Program

Cif: The CFTR Inhibitory Factor

❖ Cif is an epoxide hydrolase virulence factor that compromises airway homeostasis in CF patients.

Cif is a homodimeric epoxide hydrolase that contributes to the virulence of *Pseudomonas aeruginosa* via a bifurcated assault on airway epithelium in cystic fibrosis (CF) patients. By targeting CFTR and hydrolyzing the polyunsaturated fatty acid (PUFA) 14,15-epoxyeicosatrienoic acid (14,15-EET), Cif exacerbates the CF phenotype[1-5]. While investigating Cif's hydrolysis of host-derived substrates, we discovered side-chain rearrangements that expand the active-site volume including an open conformation of gate residues that regulate substrate access to the pocket[6]. We also developed two classes of inhibitors and high-affinity nanobodies that have proven valuable in assay development and detection of Cif[7-9]. Using techniques in enzymology and X-ray crystallography, we uncover several different cooperative and allosteric networks with implications to Cif's cell biology and drug discovery.



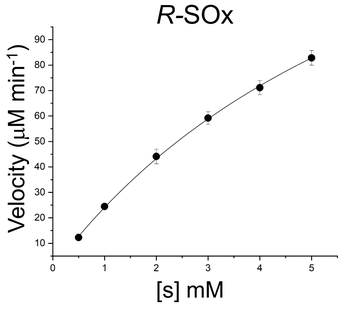
Discussion

❖ Cif hydrolysis is regulated by homotypic and heterotypic interactions in the active site and by nanobody binding at distal regions of the enzyme.

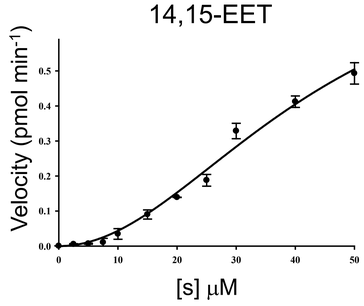
Cooperative hydrolysis of PUFAs may contribute to Cif's virulence in the lungs of CF patients where such substrates are in low abundance. It also complicates drug discovery. These interactions are to the detriment of Y2-30 where the inhibitor not only leaves half of the active-sites available to substrates, but also enhances hydrolysis! VHH108 elicits a similar response with 14,15-EET and serves as a reminder that not all immunoglobulins are beneficial. Our crystal structures provide valuable clues into how long-range communication between active sites and allosteric sites occur. Ongoing research aims to identify key residues that mediate these interactions and dissect the networks that communicate structural information from site to site.

Substrate Cooperativity

Non-Cooperative — *R*-SOx

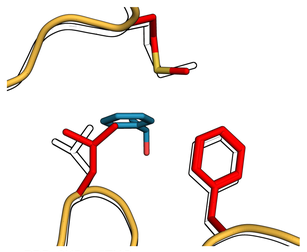


Positive Cooperativity — 14,15-EET

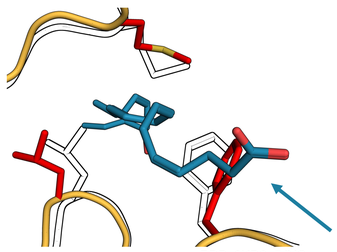


❖ Cif hydrolyzes certain substrates with positive cooperativity. These tend to be elongated PUFAs like 14,15-EET.

R-SOx Adduct



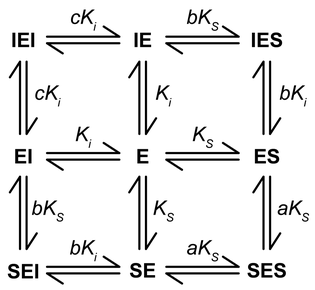
14,15-EET Adduct



Cif Adduct Apo Cif

❖ Crystal structures of Cif with trapped hydrolysis intermediates reveal a pattern of conformational rearrangements in the gate that are specific to cooperative substrates. These perturbations are caused by protrusion of the PUFA tail through the gate.

Inhibitor Cooperativity/Inhibitor-Substrate Cooperativity



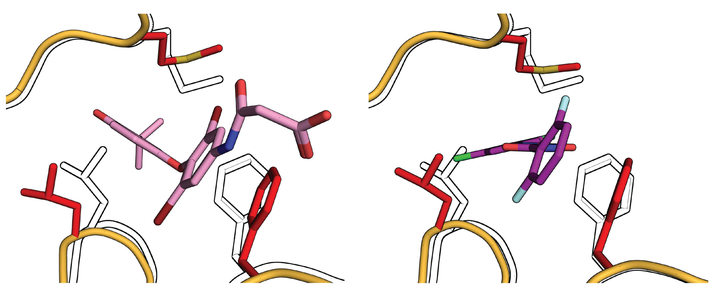
Compound	r^2	*b*	*c*	K_i (μM)	Cooperativity
Tiratricol	0.96	1	0.4 ± 0.1	2.8 ± 0.6	*c* = *positive*
KB2115	0.98	1	0.7 ± 0.2	1.4 ± 0.3	*c* = *positive*
SK510-18	0.96	1	0.3 ± 0.1	0.23 ± 0.1	*c* = *positive*
Y2-30	0.97	0.6 ± 0.1	1.9 ± 0.6	0.4 ± 0.1	*b* = positive, *c* = negative

KB2115 & SK510-18 are rationally designed derivatives of Tiratricol

❖ Interaction terms (*a, b, c*) are used as parameters in multistate, competitive system and indicate the type of cooperative interaction.

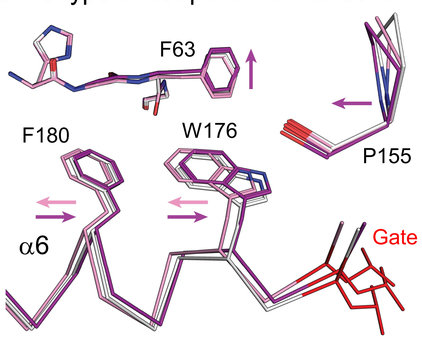
Cif Co-crystal Structures

KB2115 Y2-30



Cif KB2115 Y2-30 Apo Cif

❖ Both KB2115 and Y2-30 bind in the active site of Cif and are accompanied by shifts in the gate that resemble those that occur during hydrolysis of cooperative substrates.



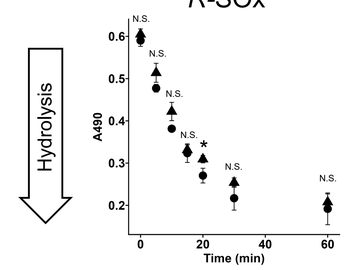
Cif bound to KB2115, Y2-30, or Apo (white)

❖ Y2-30 exerts unique orthosteric shifts on several active-site residues (e.g. F63 and P155) and along α6 at the dimer interface. KB2115 shifts α6 and interfacial side chains in the opposite direction.

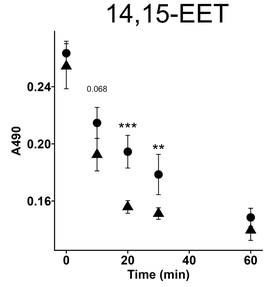
Nanobody Allostery

● Cif
▲ Cif + VHH108

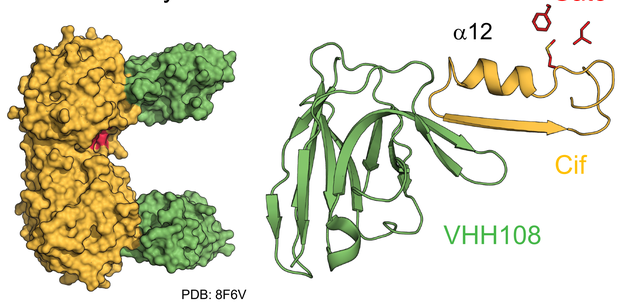
Same — *R*-SOx



Faster — 14,15-EET

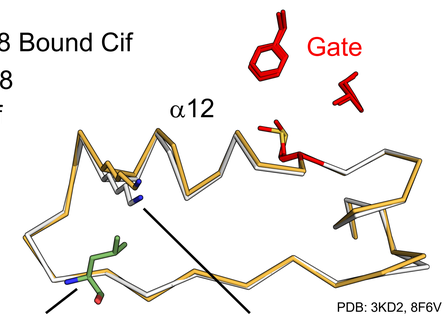


❖ VHH108 enhances Cif hydrolysis of 14,15-EET but not *R*-styrene oxide.

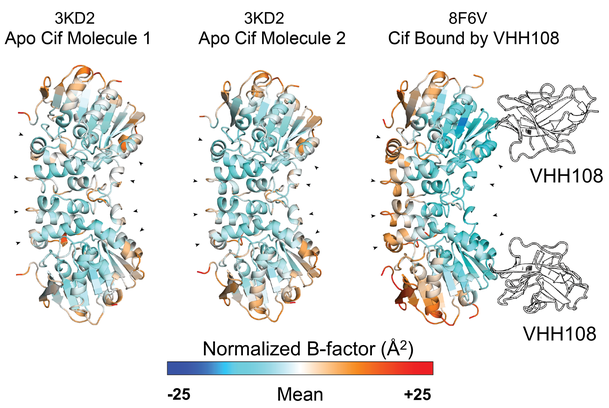


❖ VHH108 binds at the periphery of Cif with no steric interaction with the active site or gate residues.

VHH108 Bound Cif
VHH108
Apo Cif



❖ VHH108 L102 displaces Cif K281 causing a main-chain shift that propagates toward the gate.



❖ Cif experiences altered dynamics due to VHH108. This cannot be explained solely by lattice packing.

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Acknowledgements

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