# Convergent Mechanisms of Nanobody-Mediated Neutralization of the CFTR Inhibitory Factor Cif

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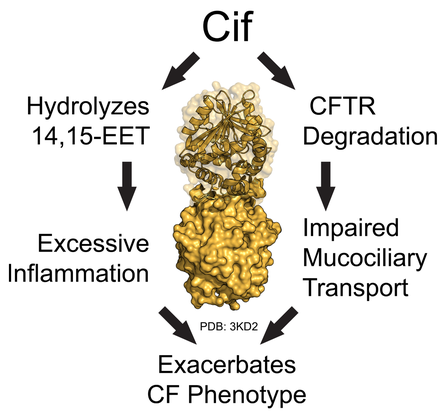
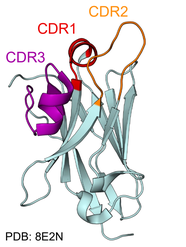
## Cif: The CFTR Inhibitory Factor




❖ Cif is a *P. aeruginosa* virulence factor that compromises airway homeostasis.

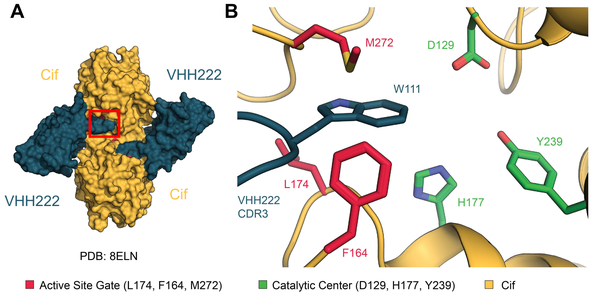
Equipped with an arsenal of virulence factors, the opportunistic airway pathogen *Pseudomonas aeruginosa* establishes chronic lung infections in cystic fibrosis (CF) patients. One of these virulence factors is Cif, a homodimeric epoxide hydrolase that causes CFTR degradation which disrupts airway homeostasis and mucociliary transport [1-3]. Cif also targets an important pro-resolving epoxide signal that regulates neutrophil transcytosis causing a hyperinflammatory environment [4,5]. This two-pronged assault exacerbates the CF phenotype [3,5].

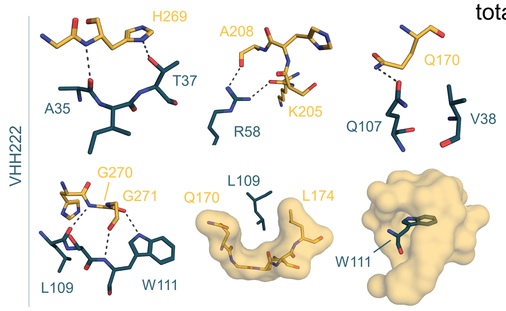



**α-Cif Nanobody VHH113**

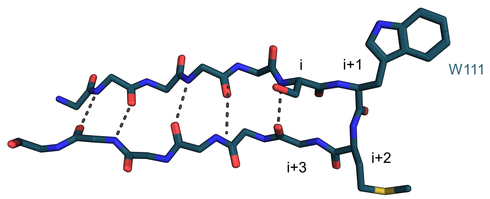
We previously developed a panel of high-affinity α-Cif VHHs and identified a subset that inhibit Cif [6]. A nanobody VHH is a single immunoglobulin domain corresponding to the heavy chain variable domain ($V_H$) of an antibody and antigen recognition occurs via three complementarity determining regions (CDRs). Using X-ray crystallography, we solved five Cif:VHH co-crystal structures and found each VHH to insert a side chain into the Cif active site in a manner suggestive of competitive inhibition. Here, we delve into these interactions to uncover how this shared mechanism is maintained across two sub-classes of neutralizing α-Cif VHHs of disparate sequence.

## 1. VHH222 Uses CDR3 to Neutralize Cif

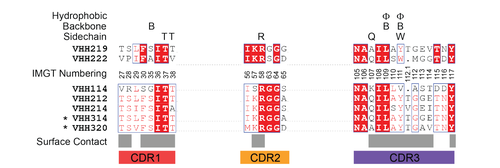



**VHH222 blocks the Cif active site.** (A) VHH222 binds near the dimer interface and overlaps with the active-site entrance. (B) A gate that opens for substrate binding is formed by F164, L174, and M272 [5]. W111 of VHH222 CDR3 inserts through this gate, blocking the active site.




**W111 is positioned for insertion into the active site.** W111 is at the corner of a Type I reverse turn with high solvent accessible surface area.




**Mapping of key interactions identifies 7 VHHs that inhibit Cif using a hydrophobic sidechain (W/Y/V) in CDR3.** Specific interactions between Cif:VHH222 and Cif:VHH219 were mapped to all α-Cif VHHs to identify related sequences predicted to function like VHH222. Asterisks (*) mark sequences that failed initial screening but were verified to inhibit Cif following this analysis.

### Acknowledgements

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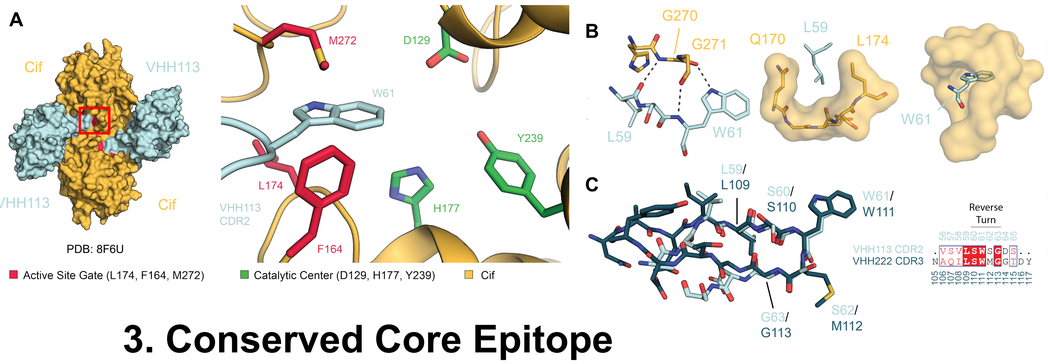
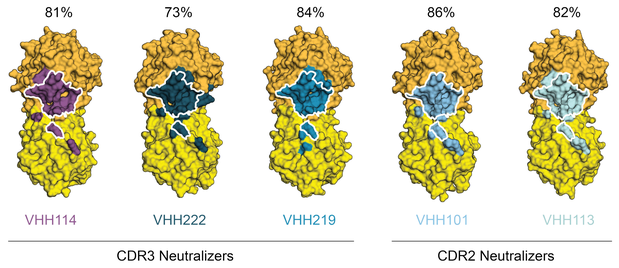
## 2. VHH113 Uses CDR2 to Neutralize Cif



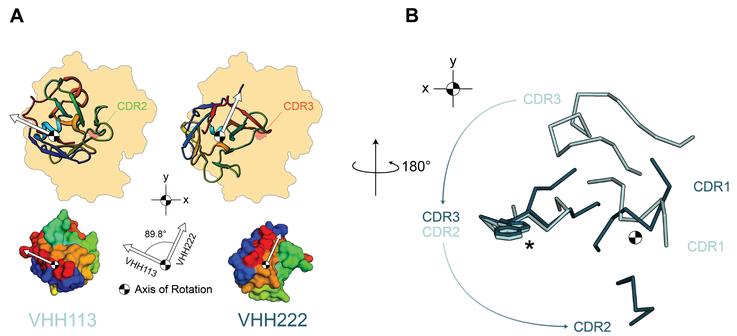

**An analogous Type 1 reverse turn in VHH113 CDR2 mimics VHH222 CDR3.** (A) VHH113 resembles VHH222 in terms of complex formation and neutralization. (B) Contacts between Cif and VHH113 CDR2 are nearly identical to those of Cif and VHH222 CDR3. (C) A region spanning the reverse turn and preceding Leu in VHH222 CDR3 is conserved in VHH113 CDR2. These reverse turns overlap following alignment of the co-crystal structures to apo Cif by main-chain atoms of Cif.

## 3. Conserved Core Epitope



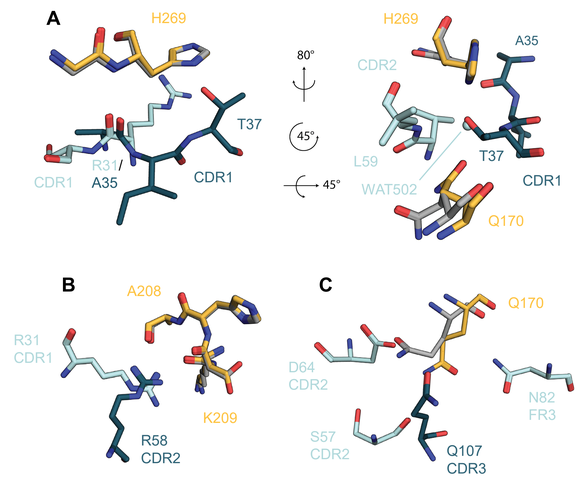

**VHHs footprint to the same region of Cif and share a "core epitope."** Epitopes were mapped to Cif and we identified a subset of residues that participate in all Cif:VHH interactions. We refer to this as a "core epitope," demarcated by the white outline. The percentage contribution of the core epitope to the total epitope is displayed above each epitope map.

## 4. Paratope Mimicry




**Orientation of VHH113 and VHH222 is offset by an 89.8° rotation when bound to Cif.** The viewing angle is along the rotation axis (black and white pinwheel). (A) VHH113 and VHH222 bind to the same region of Cif but are rotated relative to one another by 89.8°. Cif is depicted as an orange-filled outline with the active site in red. VHHs are colored rainbow from N- to C- terminus and white arrows are a visual aid to track rotation. (B) The CDRs rotate such that VHH113 CDR2 takes the place of VHH222 CDR3. CDR1 is near the rotation axis and remains in the same approximate location, but in different orientations. The Trp sidechain marked with an asterisk (*) gets inserted into the active site.

## 5. Convergent VHH Interactions




**Convergent Cif:VHH interactions between VHH113 and VHH222.** Despite having largely unrelated paratopes, both VHHs interact with Cif through the "core epitope." Due to rotation of one VHH relative to the other, residues in different CDRs preserve these interactions. These VHHs therefore recognize nearly identical, highly overlapping epitopes.

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## Discussion

All neutralizing VHHs from our library share an analogous mechanism of Cif inhibition where a hydrophobic sidechain (W/Y/V) at position i+1 of a Type I reverse turn inserts through the active site gating mechanism to block access. This reverse turn is part of CDR3 or CDR2 giving rise to two sub-classes. VHHs of both sub-class footprint to the same region of Cif and share a "core epitope" accounting for 73% - 86% of the total epitope. However, these sub-classes bind to Cif with an approximate 90° rotation that drastically alters the spatial localization of equivalent CDRs relative to one another. Despite having structurally unique paratopes, both VHH sub-classes interact with the same Cif residues. In consideration of the observed Cif:VHH interactions and the mechanism of inhibition, we conclude these VHH paratopes are an example of conformational equivalence. The preference for a "core epitope" also provides insight into antigenic hotspots found in antigen:nanobody interactions.

### References

1. Bomberger et al. (2011) *PLoS Pathog.* **7**:3
2. Bahl et al. (2010) *J Bacteriol.* **192(7)**:1785
3. Hvorecny et al. (2018) *Am J Physiol Lung Cell Mol Physiol.* **314**:L150
4. Hvorecny et al. (2017) *Structure.* **25(5)**:697
5. Flitter et al. (2017) *Proc Natl Acad Sci U S A*
6. Vasylieva et al. (2019) *Anal Chim Acta.* 2019 **1057**:106.