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Does VH298 glue CDO1 to VHL?

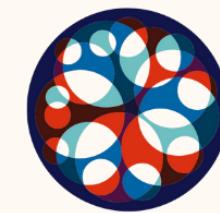
My 6-week summer project at the CeTPD

Sansara Klinsukont; 2025 Summer Student





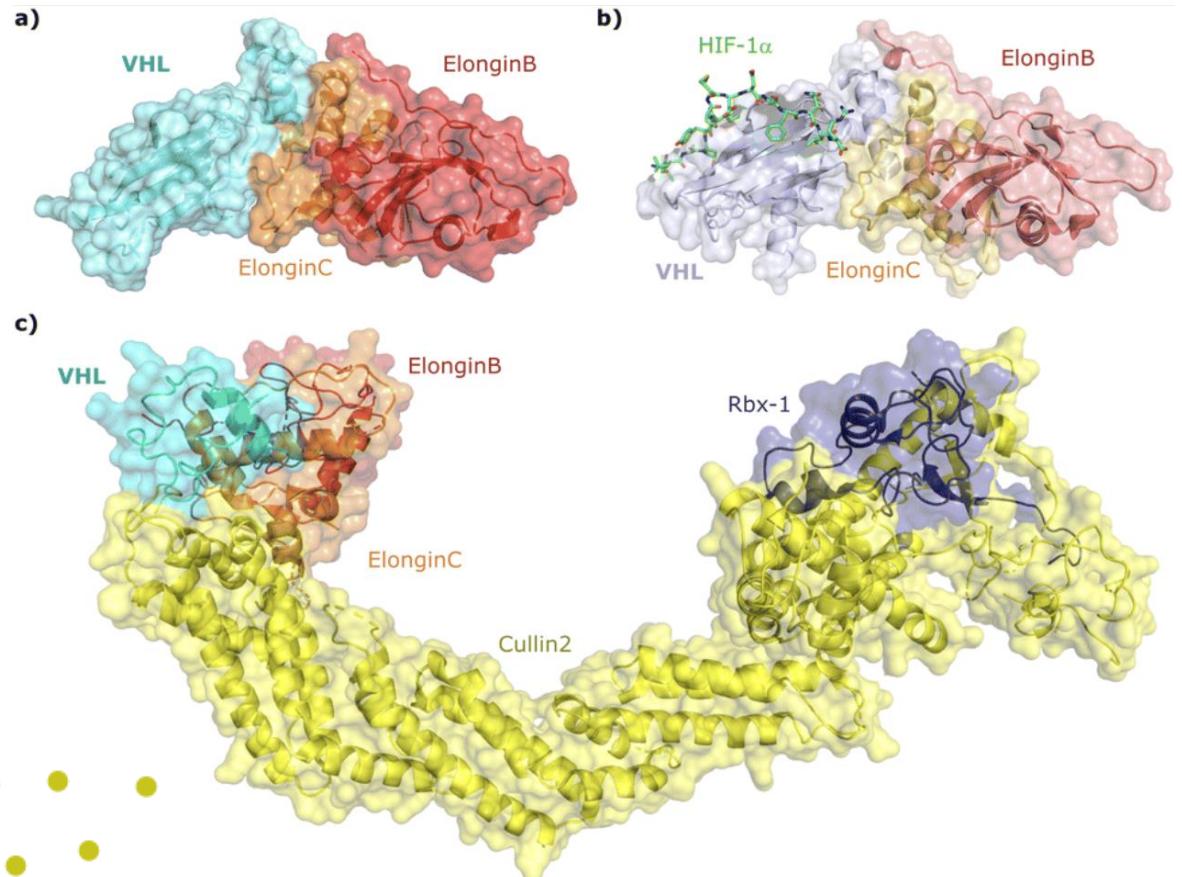
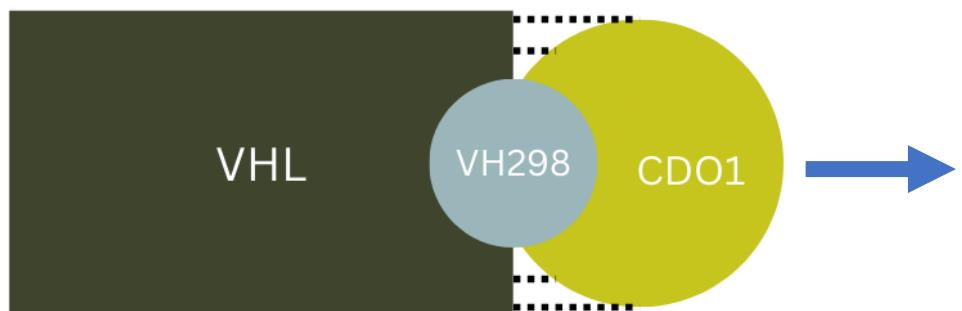
A little about me



**BIOCHEMICAL
SOCIETY**



Background



(J. Diehl and A. Ciulli, 2022)



Project aim

To learn more about the **effects of VH298 on the interactions between VCB and CDO1.**

Express and purify
VCB



Express and purify CDO1
and CDO1 (^{15}N)



Structural analysis by
ITC and NMR





Workflow

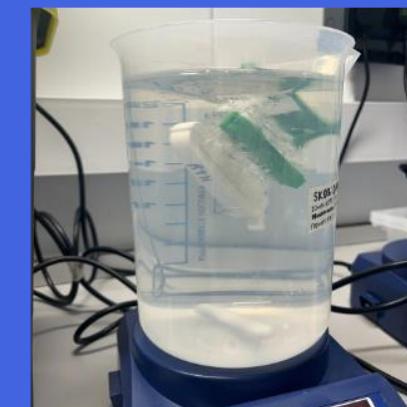
Express and purify VCB

Grow transformed *E. coli* in LB. Lyse cells and purify via HisTrap → Reverse HisTrap → AEC → SEC



Express and purify CDO1 and CDO1 (N)

Grow transformed *E. coli* in M9. Lyse cells and purify via GST-Trap and SEC.



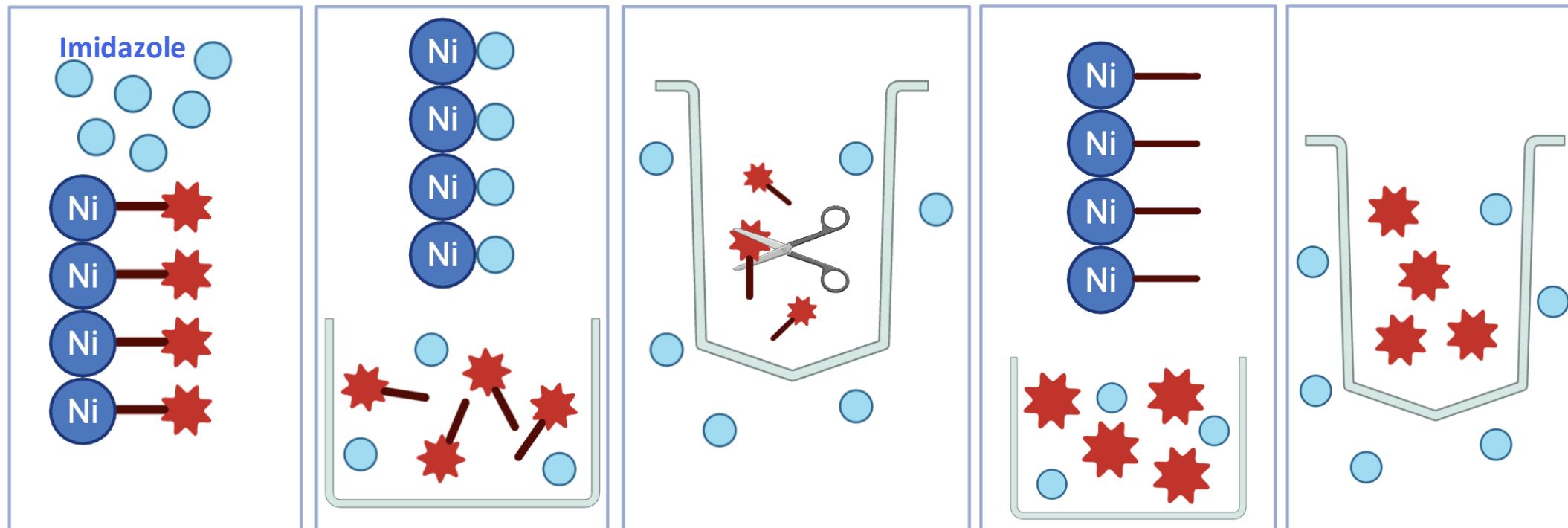
Structural analysis – NMR only

ITC machine unavailable. Used NMR to validate effect of VH298 on VCB-CDO1 glueing.



Workflow

VCB His-Trap and Reverse His-Trap



HisTags bind to Nickel beads, other proteins wash off

Imidazole competes with HisTags, VCB washes off

His-Tags cleaved off with TEV, imidazole removed via dialysis

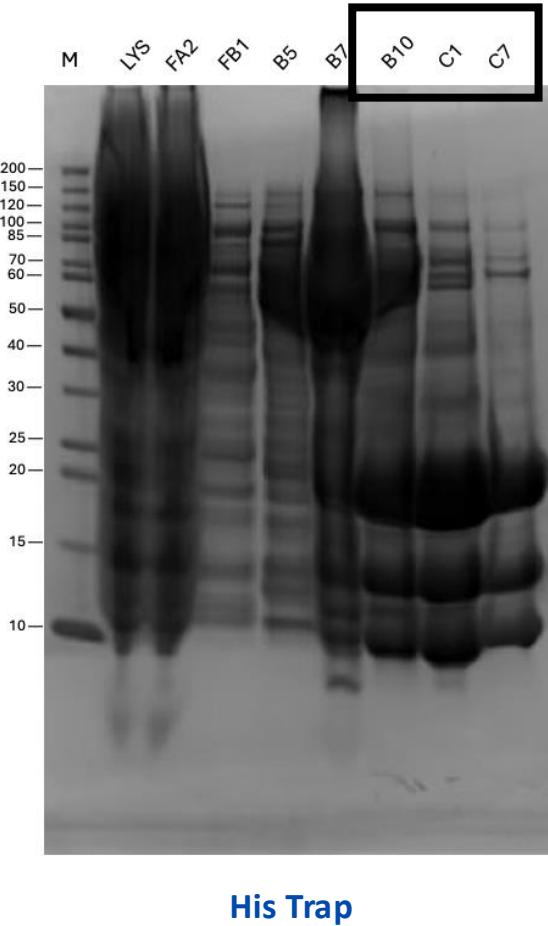
Second His-Trap, only free-Histags bind, VCB flows through

Imidazole removed via dialysis



Workflow

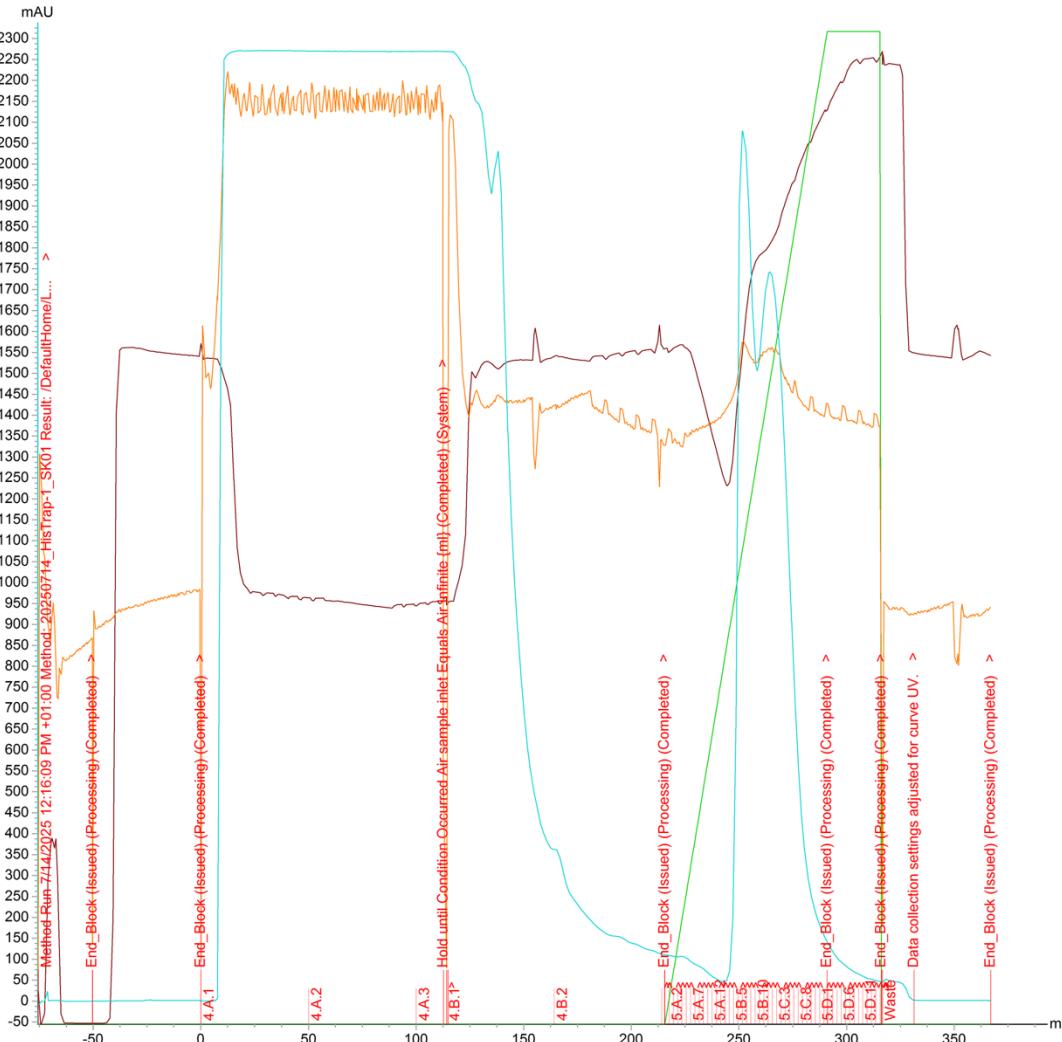
VCB His-Trap and Reverse His-Trap



VHL
Elongin B
Elongin C

Cond_Chrom.1:20250714_HisTrap-1_SK01_001.001
PreC_pressure_Chrom.1:20250714_HisTrap-1_SK01_0...
Injection_Chrom.1:20250714_HisTrap-1_SK01_001.001
Run Log_Chrom.1:20250714_HisTrap-1_SK01_001.001

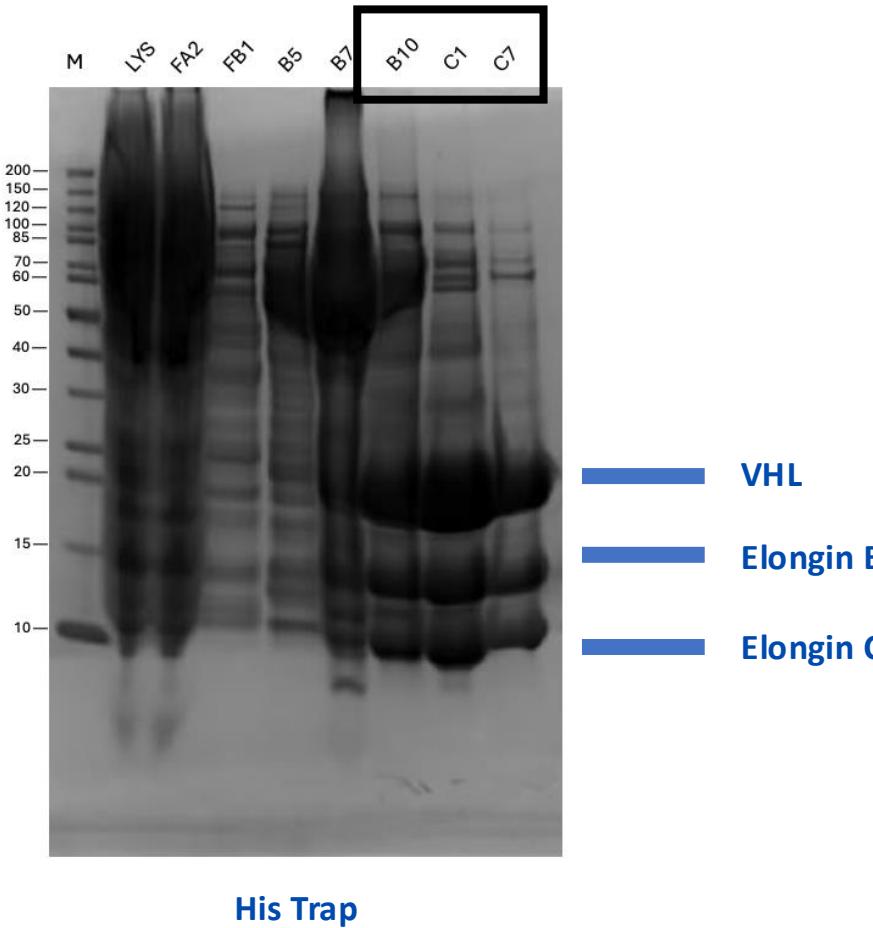
Conc_B_Chrom.1:20250714_HisTrap-1_SK01_001.001
Fraction_Chrom.1:20250714_HisTrap-1_SK01_001.001
UV_Chrom.1:20250714_HisTrap-1_SK01_001.001





Workflow

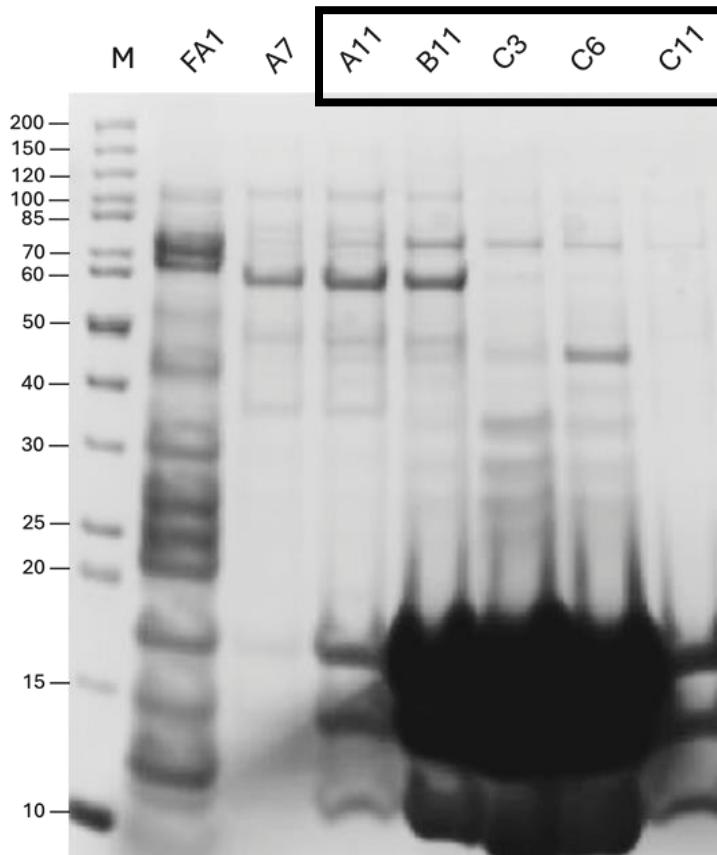
VCB His-Trap and Reverse His-Trap



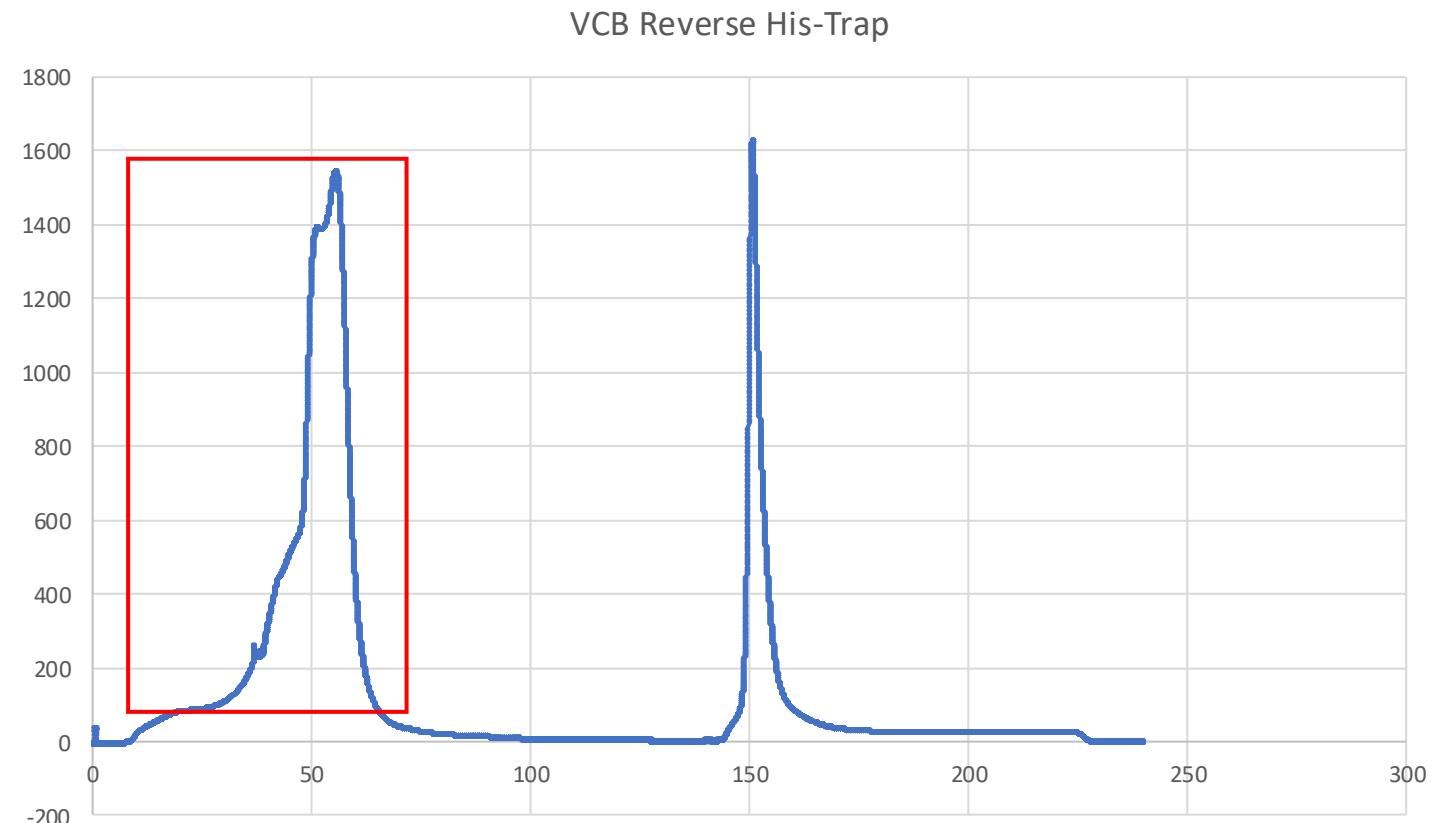


Workflow

VCB His-Trap and Reverse His-Trap



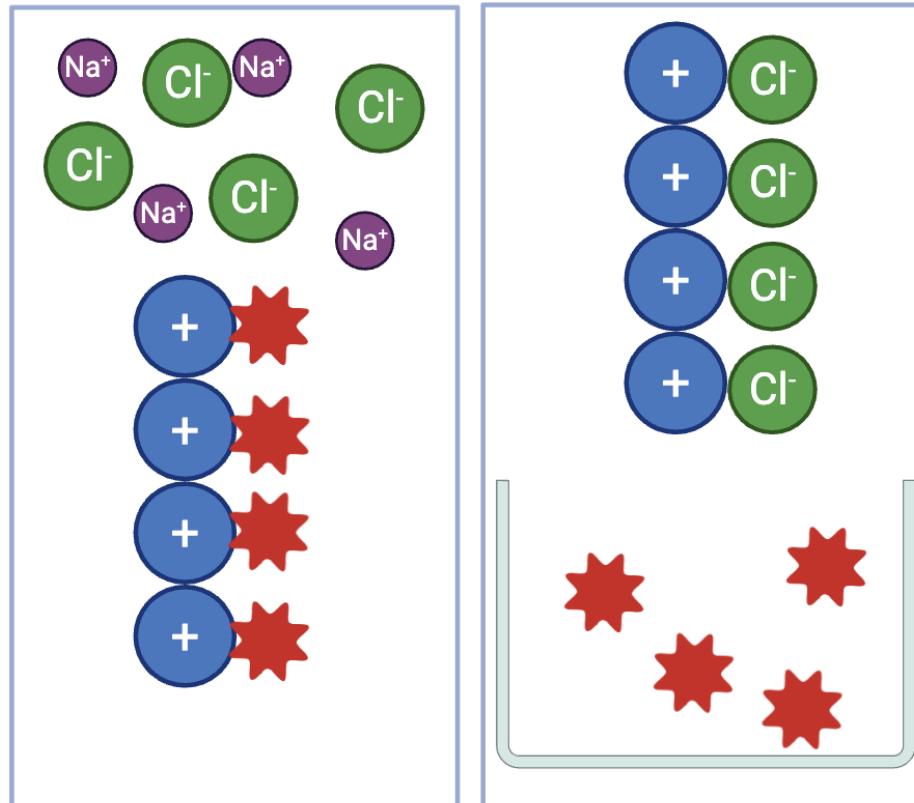
Reverse His-Trap





Workflow

VCB Anion-Exchange Chromatography (AEC)



VCB is negatively charged at buffer pH (7.0) and binds to positive beads in the column.

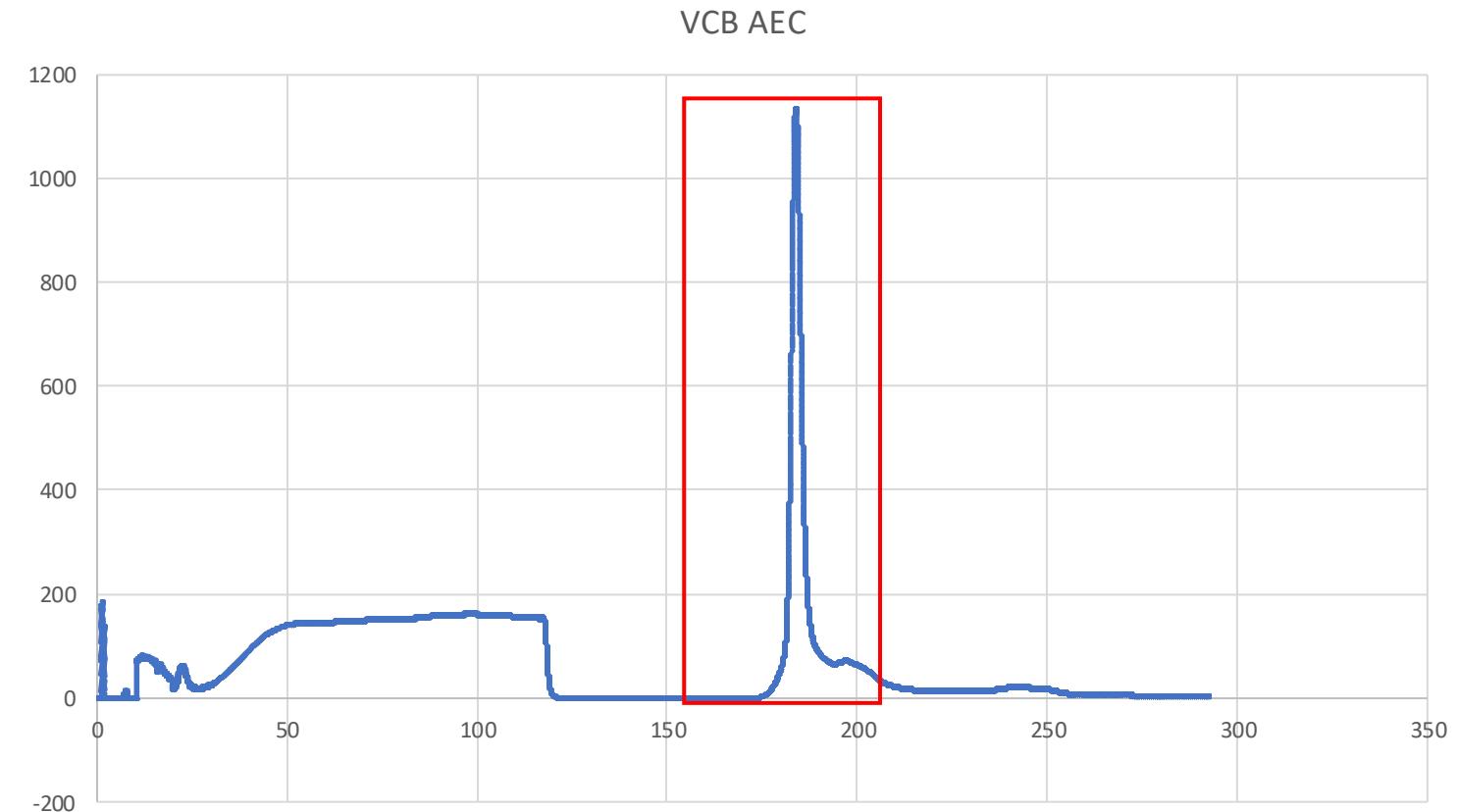
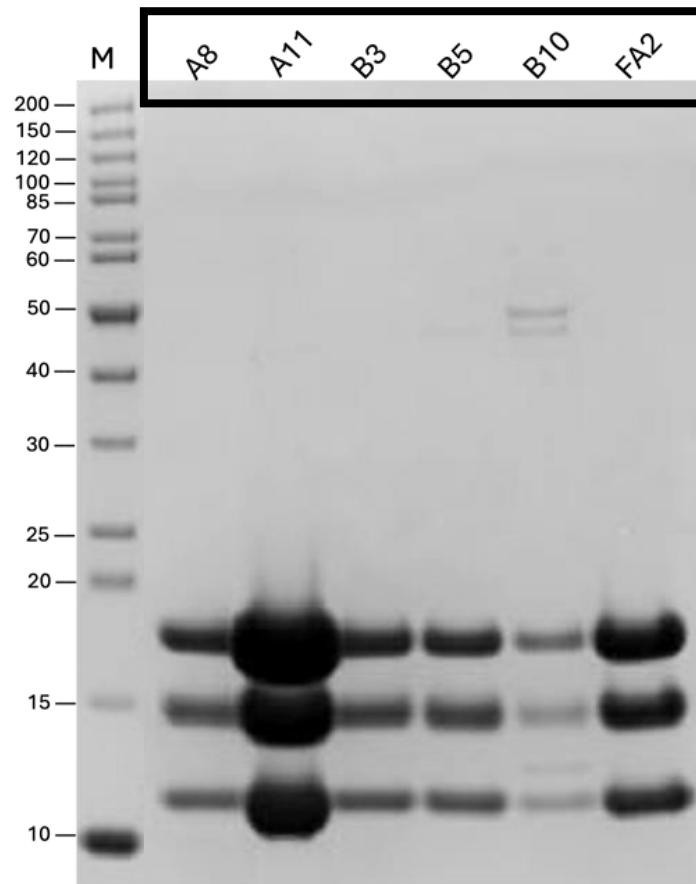
Elute with high conc. NaCl, Cl⁻ displaces bound VCB

VCB washes off.



Workflow

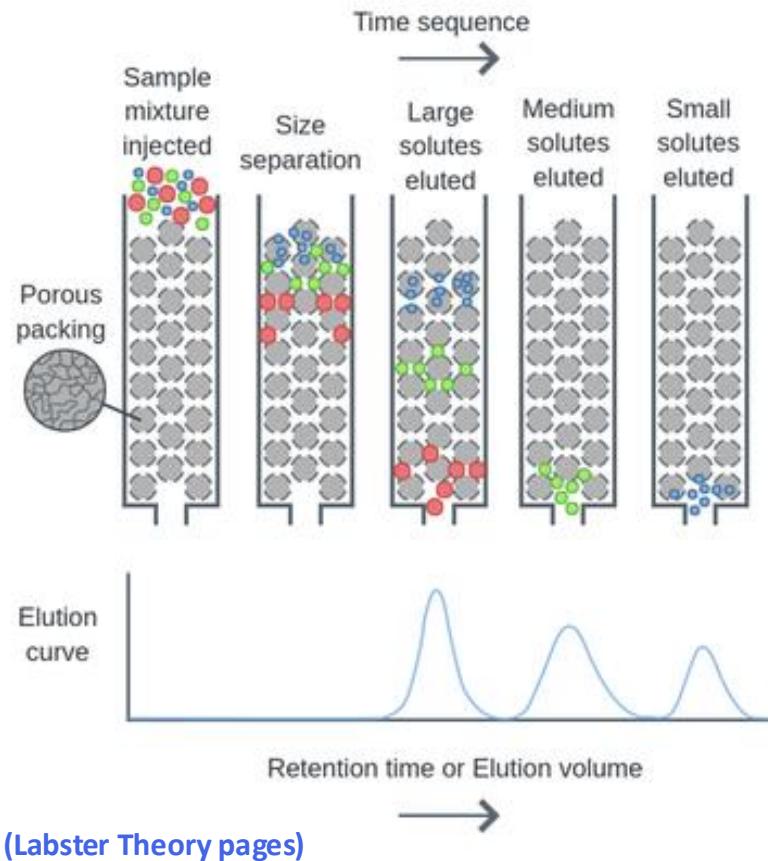
VCB Anion-Exchange Chromatography (AEC)





Workflow

VCB Size-Exclusion Chromatography (SEC)



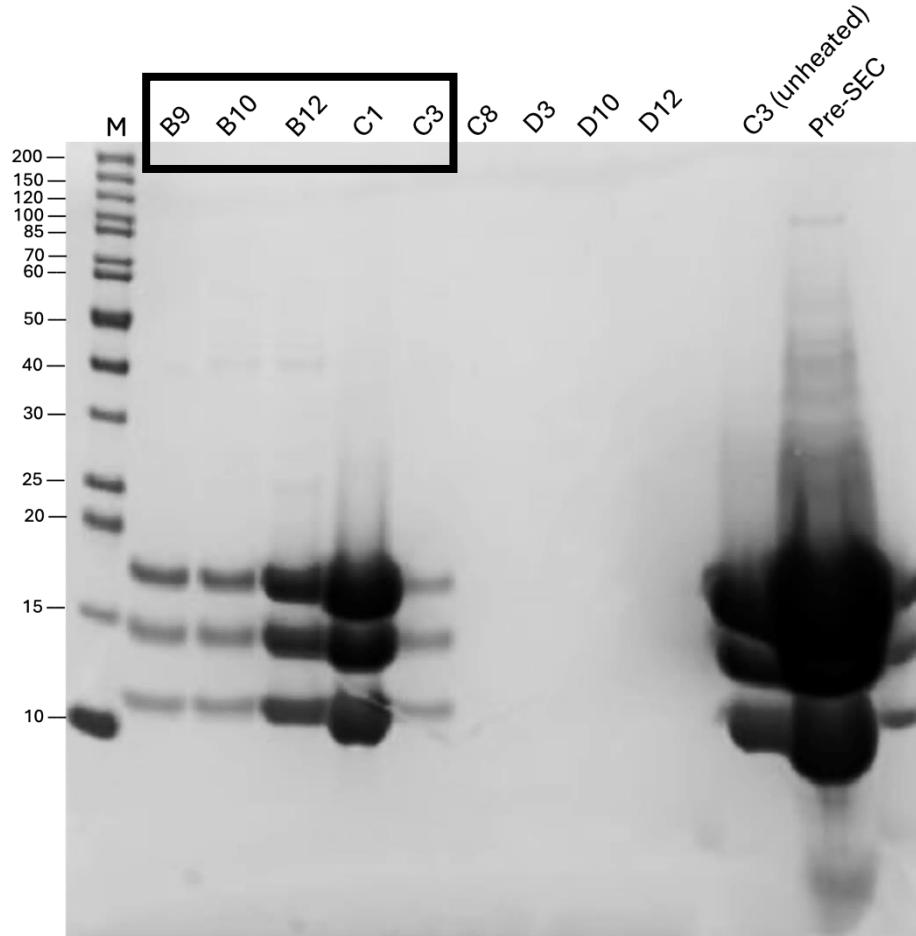
VCB enters pores of resin beads while other larger proteins flow through.

VCB eventually flows through the column and is collected



Workflow

VCB Size-Exclusion Chromatography (SEC)



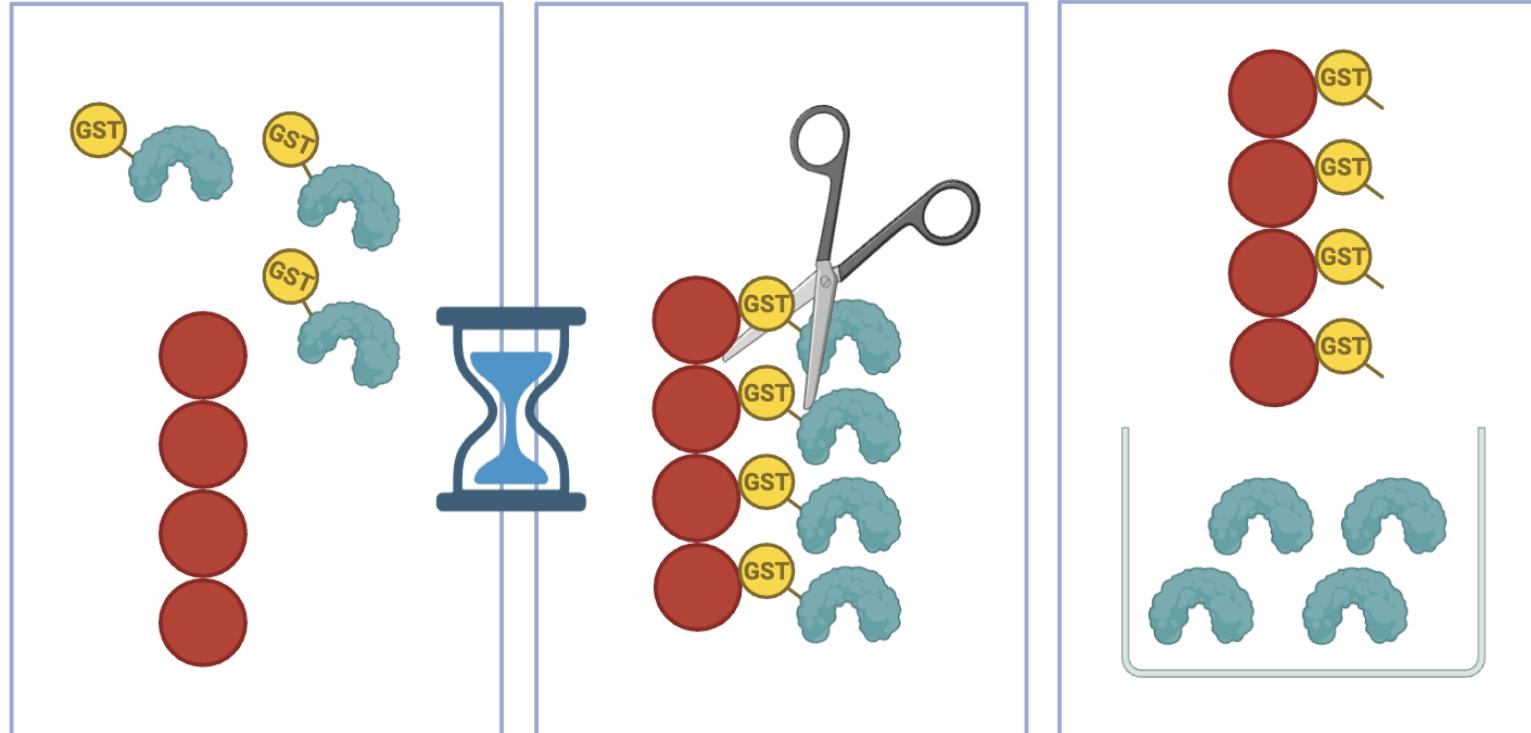
VCB concentration

- 52.6 mg/mL
- 1.27 mM
- ~400 µL total



Workflow

CDO1 GST-Trap (Gravity column)



GST-Tags bind to Glutathione beads.

Allow binding, wash off unbound protein.

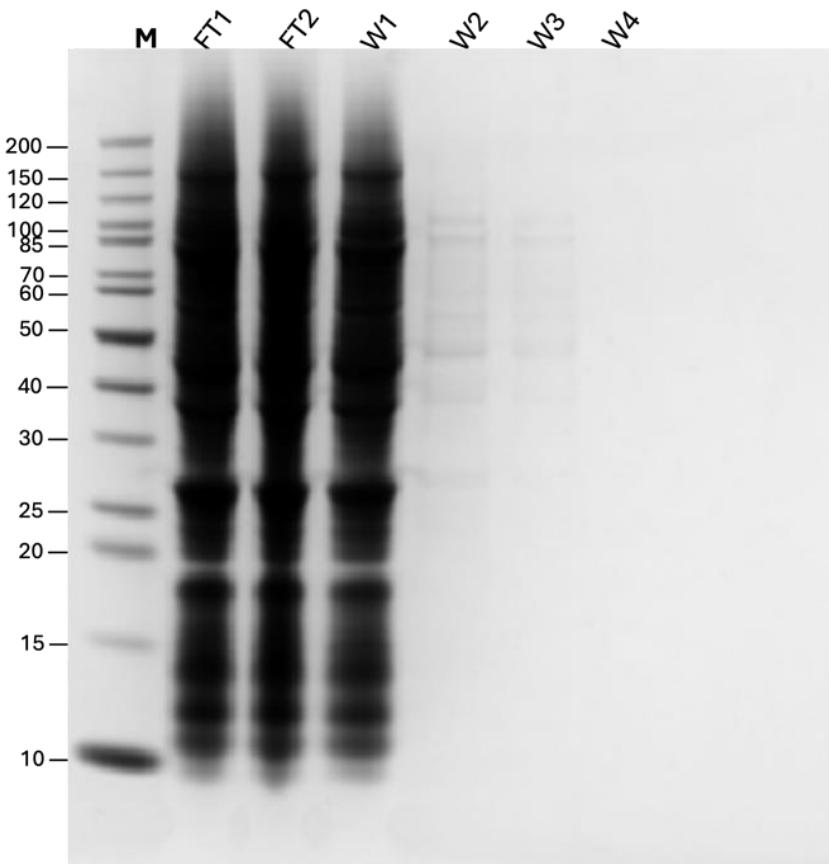
Cleave tag overnight (precision protease).

Wash column to retrieve CDO1.

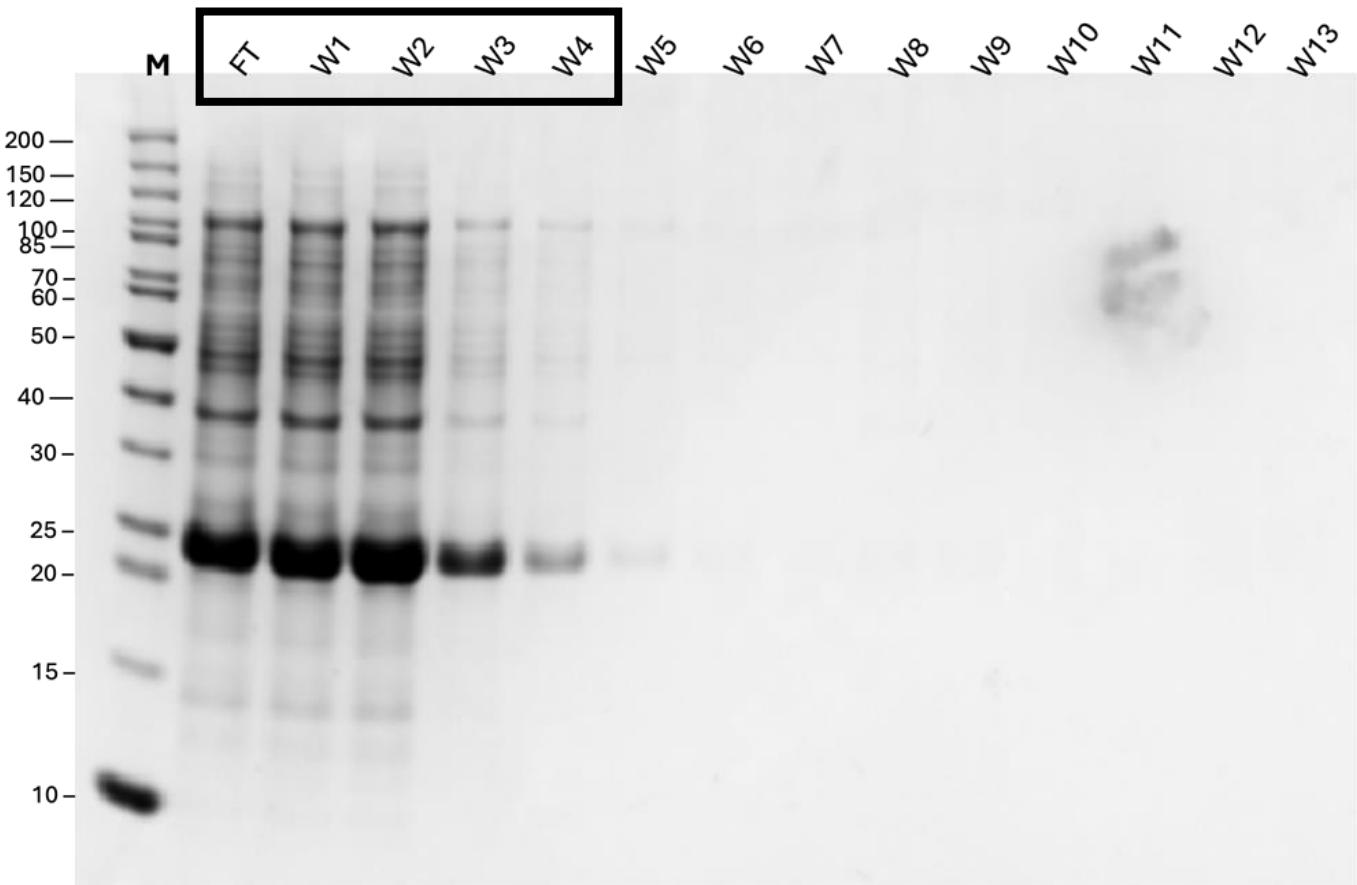


Workflow

CDO1 GST-Trap



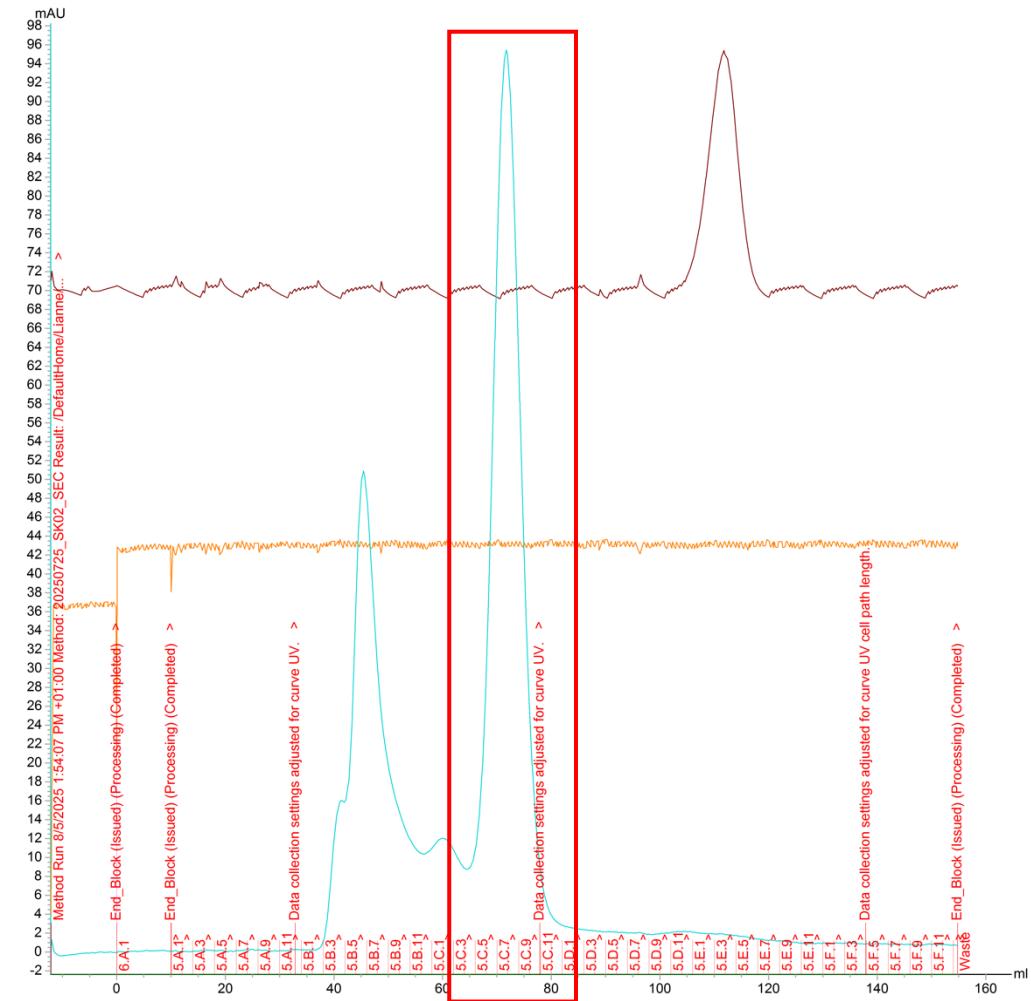
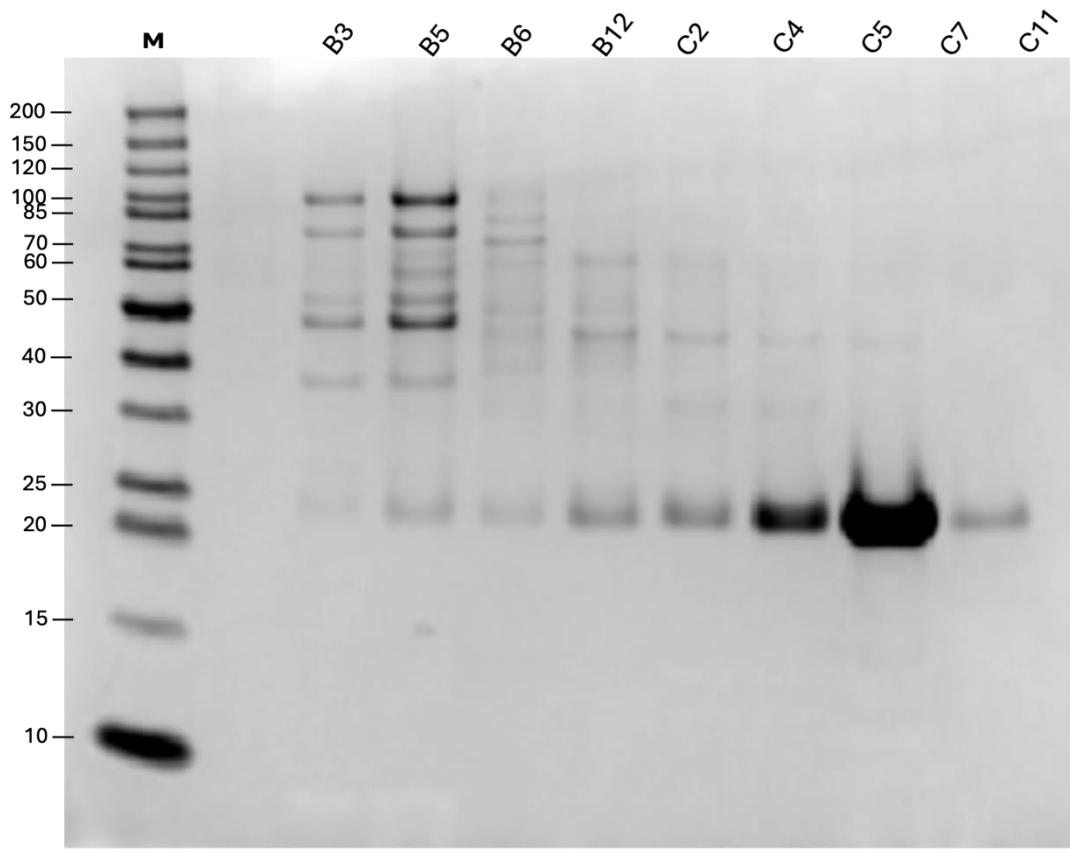
GST-Trap: Initial wash



GST-Trap: Washes after binding and cleaving

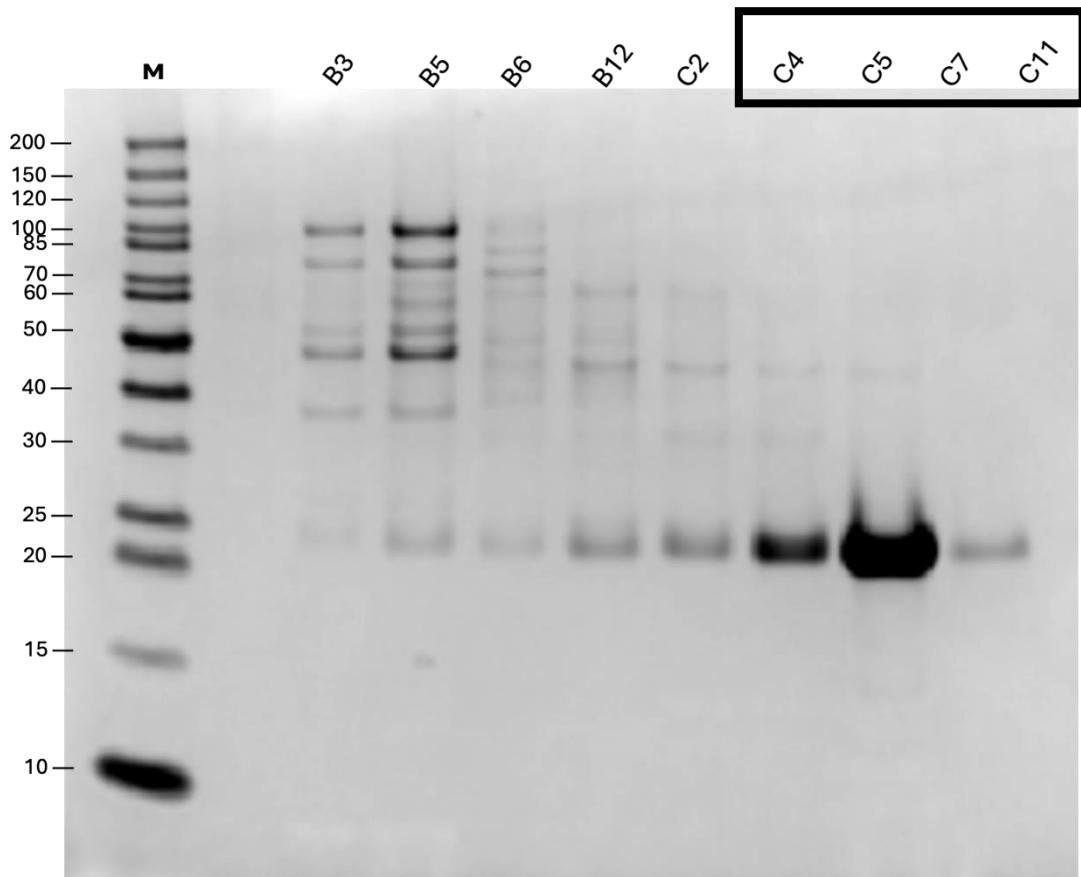


Results: ^{15}N Labelled CDO1 (SEC)





Results: ^{15}N Labelled CDO1 (SEC)



^{15}N CDO1 concentration

- 33.27 mg/mL
- 1.38 mM
- ~100 μL total

CDO1 concentration

- 8.74 mg/mL
- 0.37 mM
- ~1500 μL total



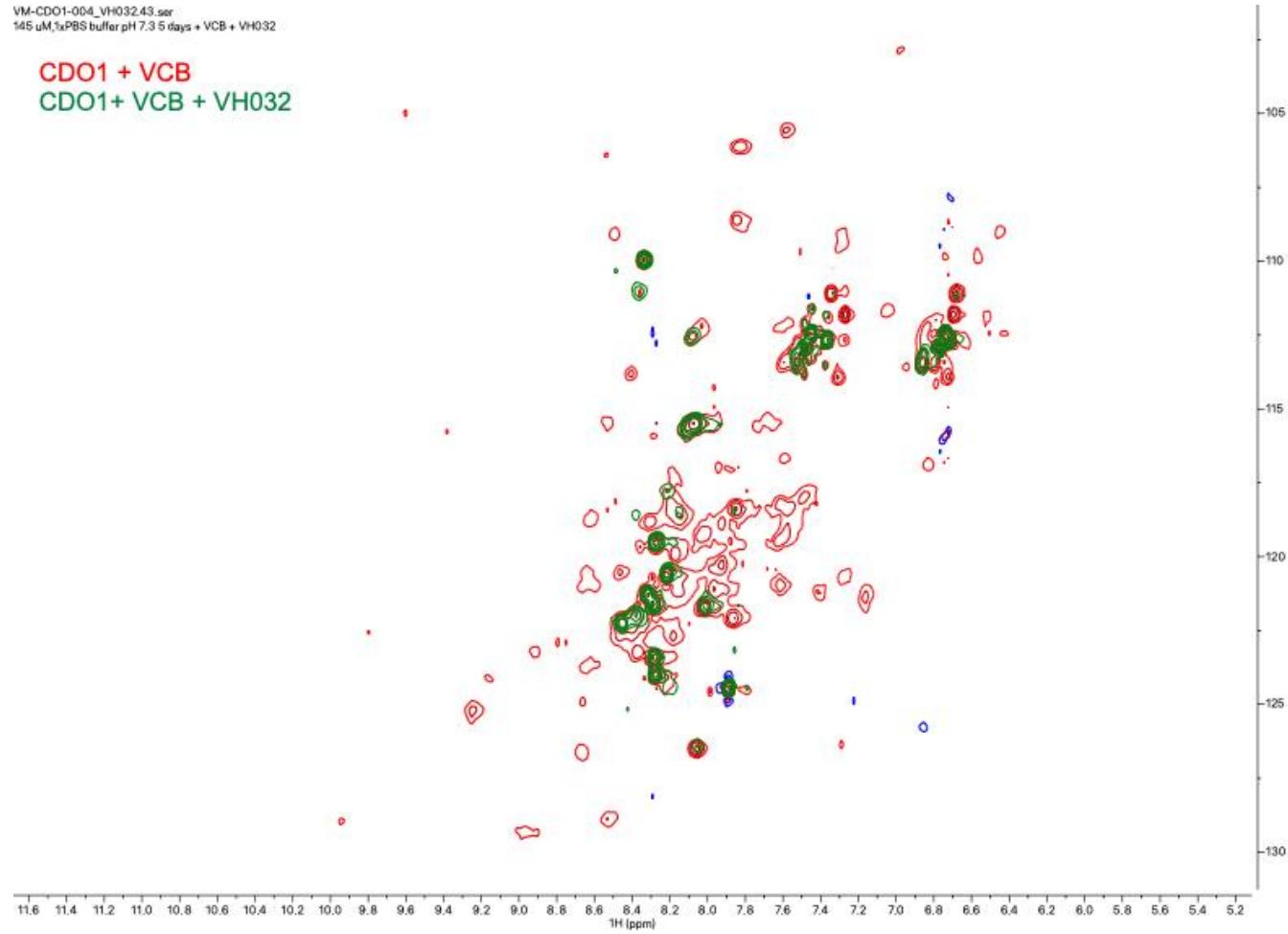
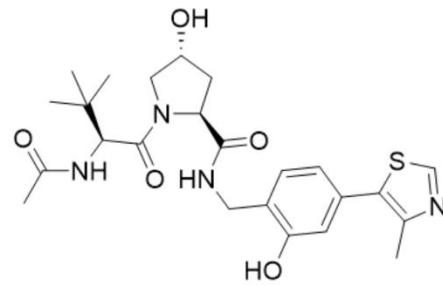
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VH032

VM-CDO1-004_VH032.43.ser
145 μM; 1% PBS buffer pH 7.3 5 days + VCB + VH032

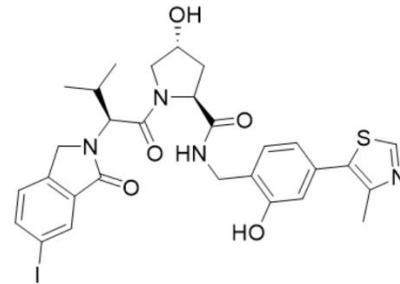
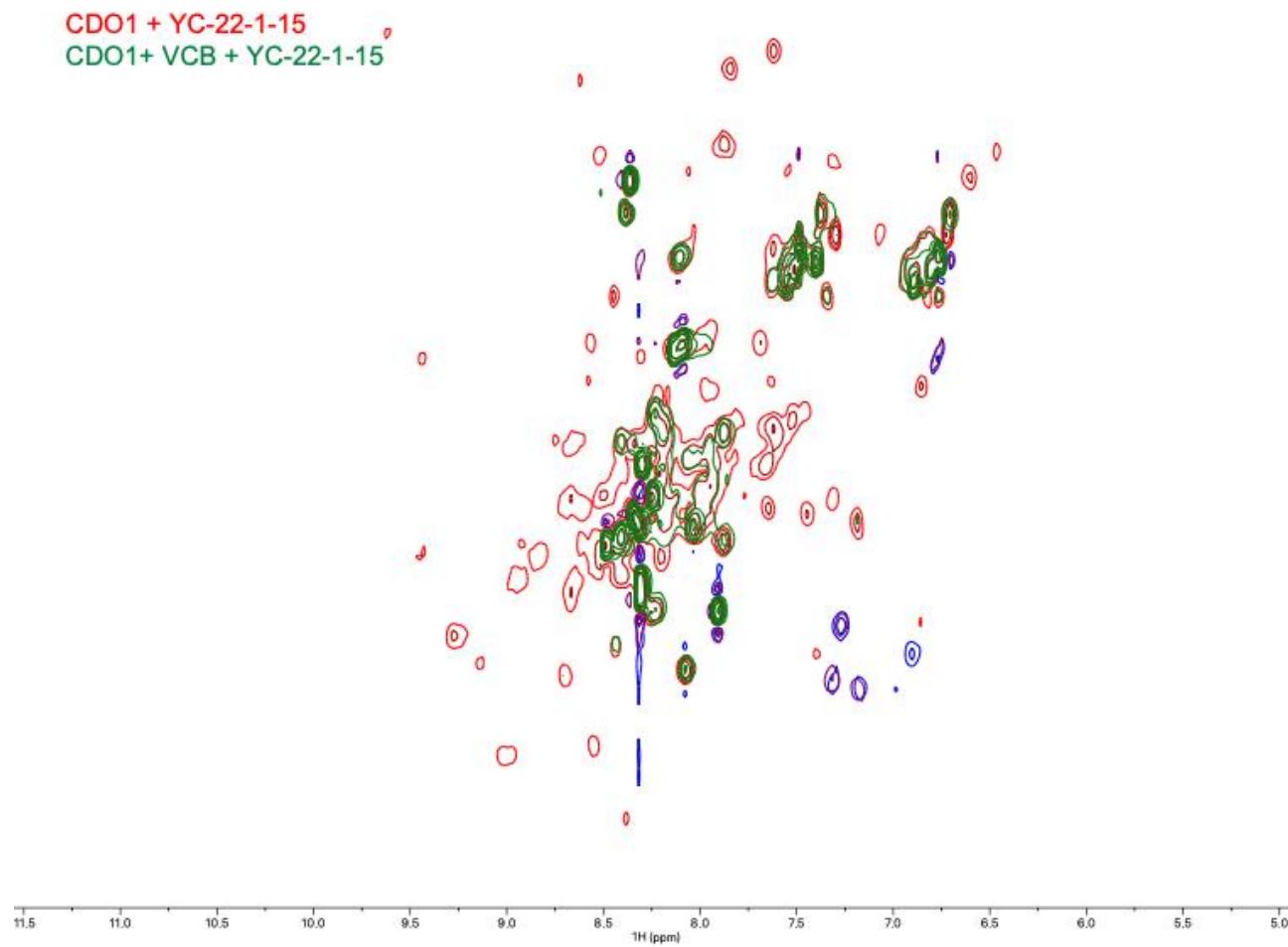
CDO1 + VCB
CDO1+ VCB + VH032



CDO1 + VCB vs. CDO1 + VCB + VH032



YC-22-1-15

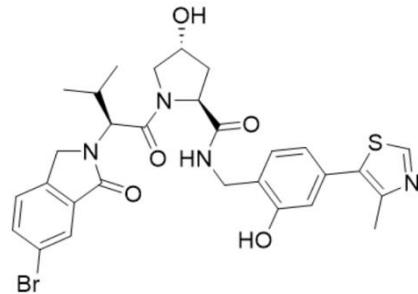


CDO1 + YC-22-15 vs. CDO1 + VCB + YC-22-15

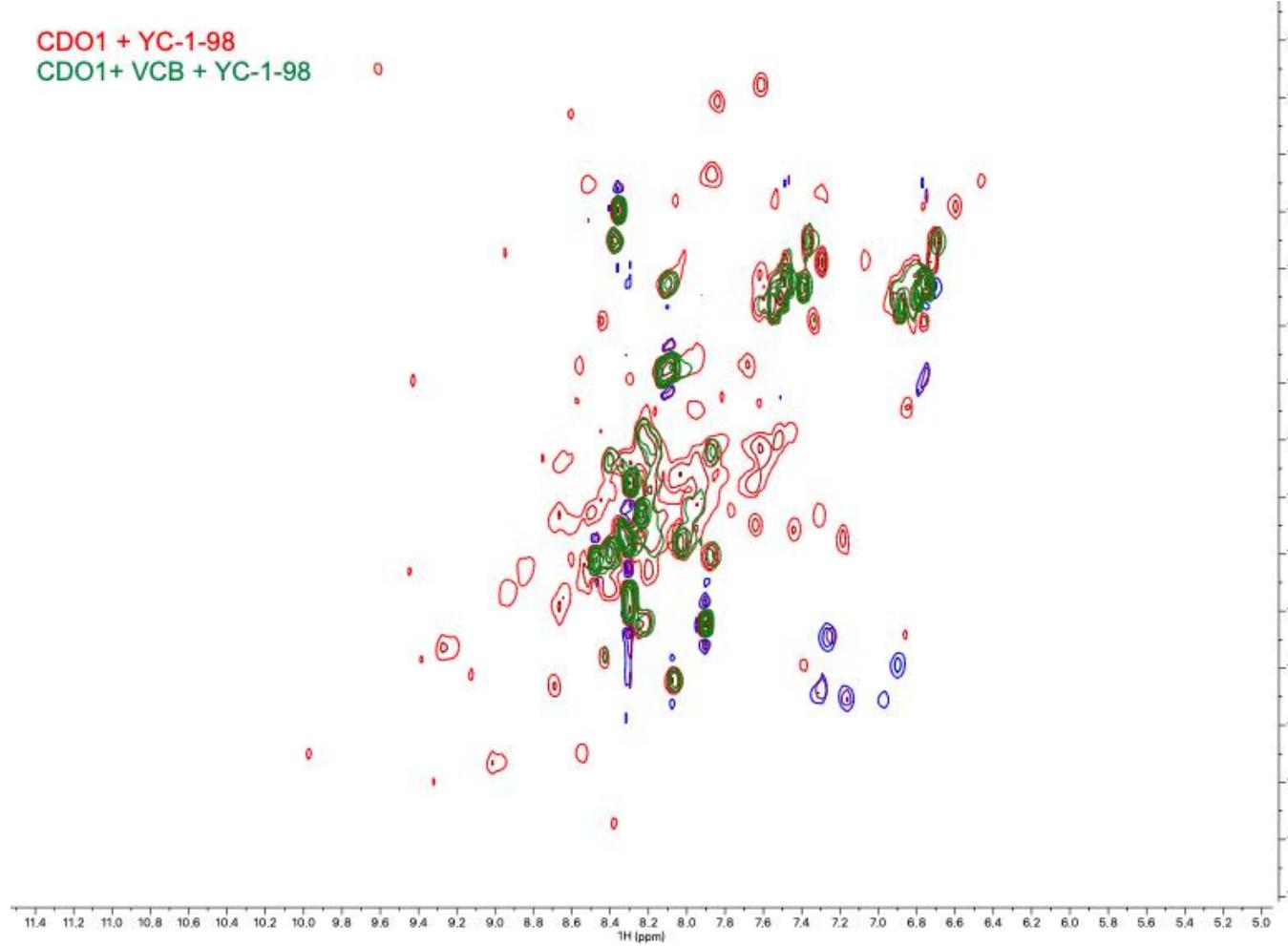




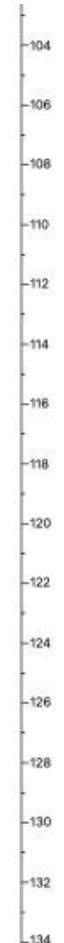
YC-1-98



CDO1 + YC-1-98
CDO1+ VCB + YC-1-98



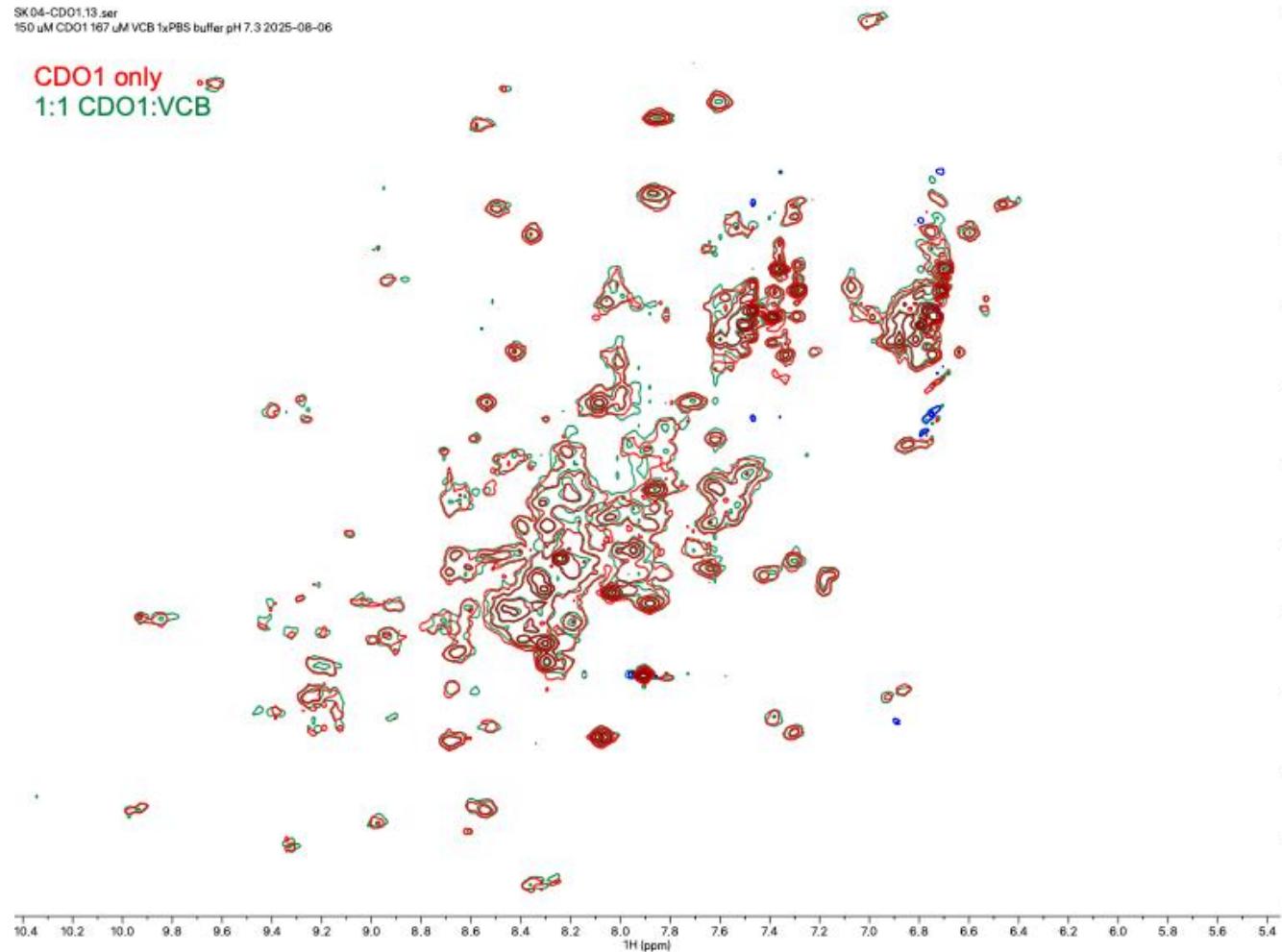
CDO1 only vs. CDO1 + VCB + VH032





SK04-CDO1.13.ser
150 μ M CDO1 167 μ M VCB 1xPBS buffer pH 7.3 2025-08-06

CDO1 only
1:1 CDO1:VCB

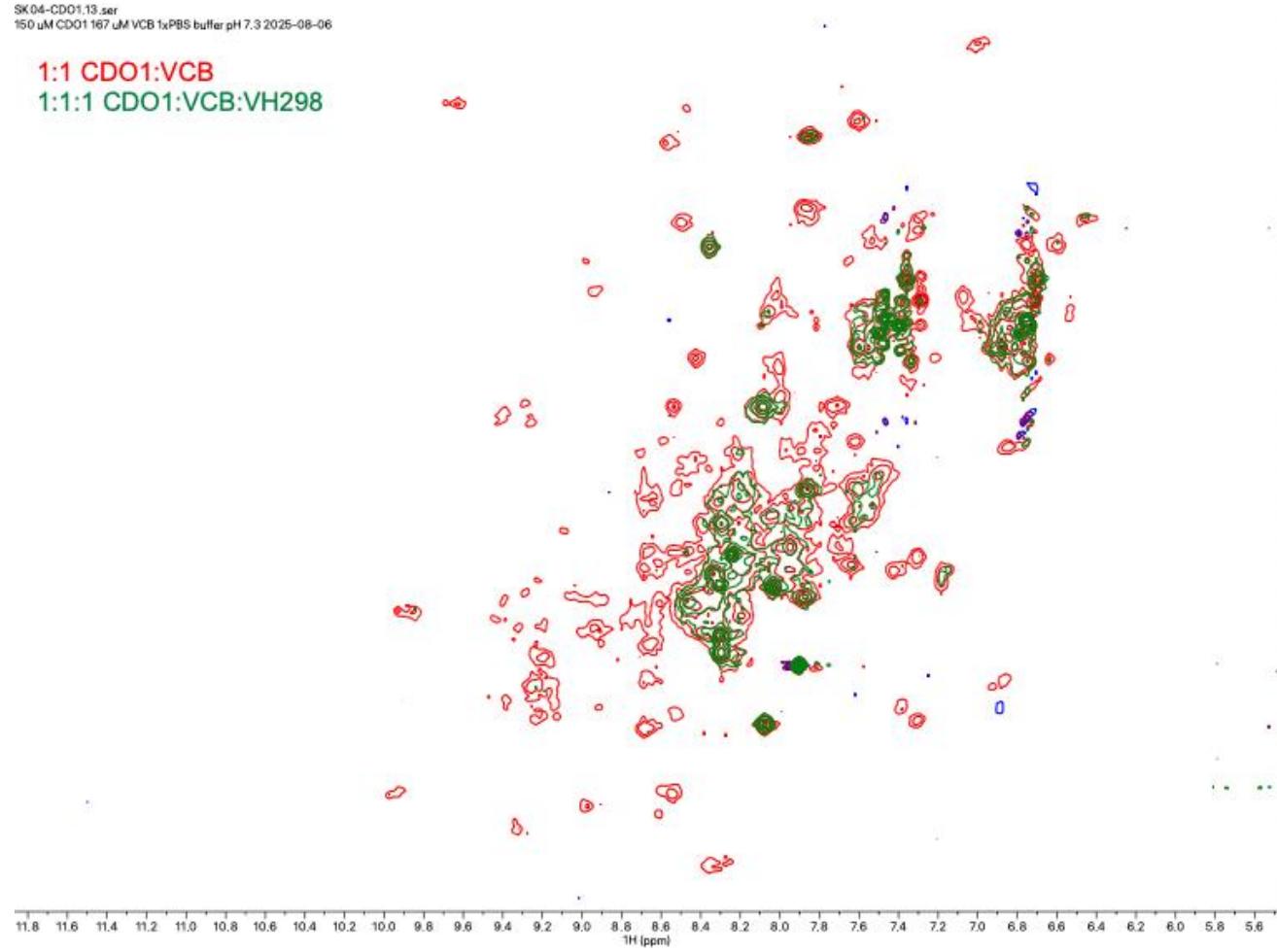


CDO1 only vs. 1:1 CDO1:VCB

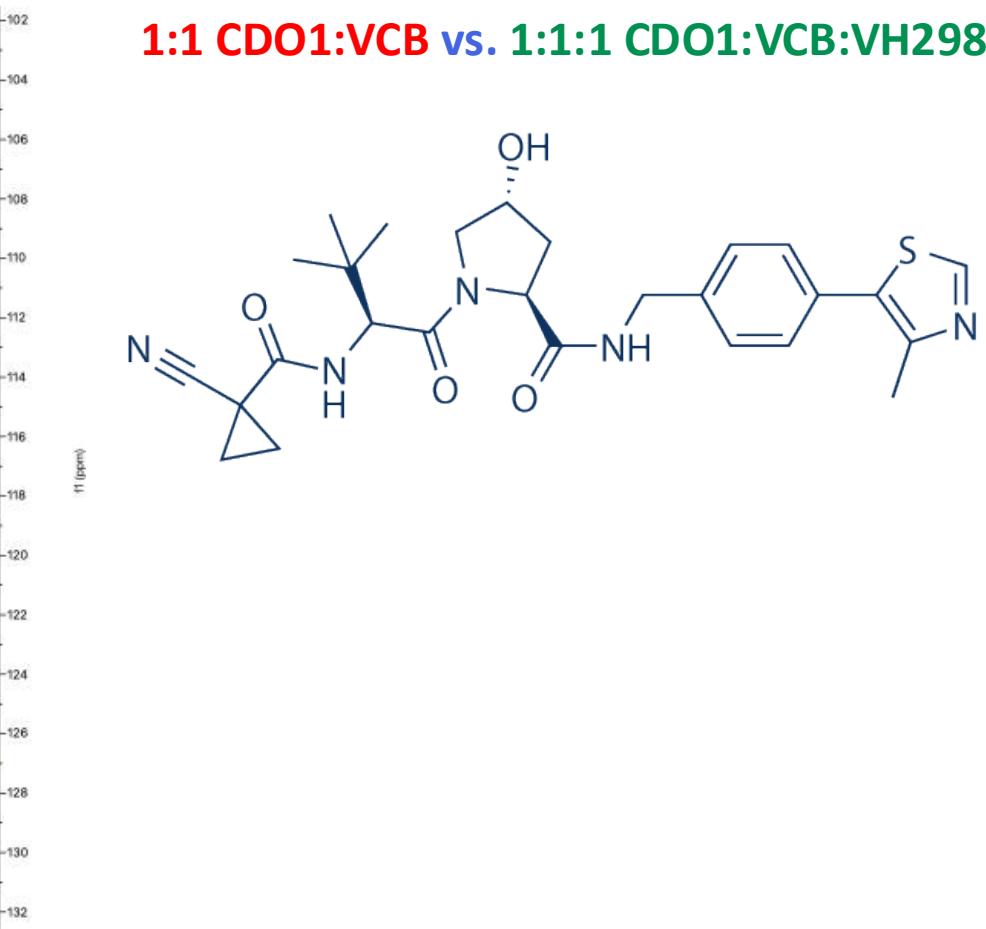
VH298

SK04-CDO1.13.ser
150 uM CDO1 167 uM VCB 1xPBS buffer pH 7.3 2025-08-06

1:1 CDO1:VCB
1:1:1 CDO1:VCB:VH298



1:1 CDO1:VCB vs. 1:1:1 CDO1:VCB:VH298





SK04-CDO1.13.ser
150 μ M CDO1 167 μ M VCB 1xPBS buffer pH 7.3 2025-08-06

1:1 CDO1:VCB
1:3 CDO1:VCB

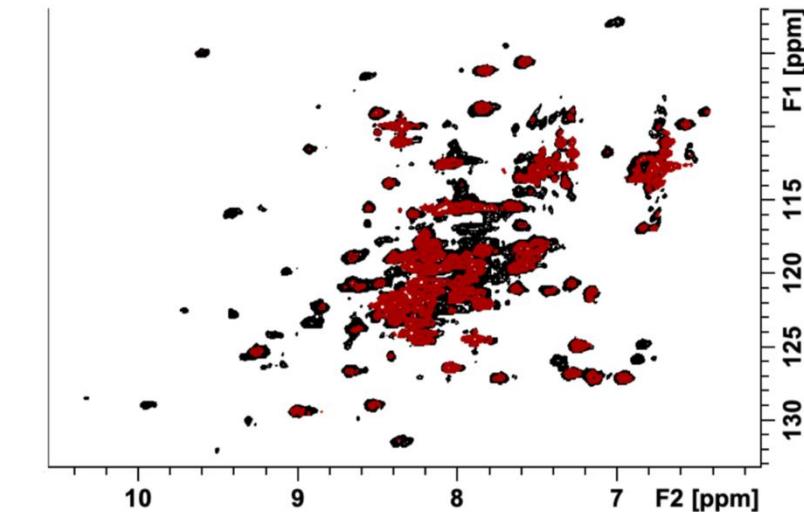
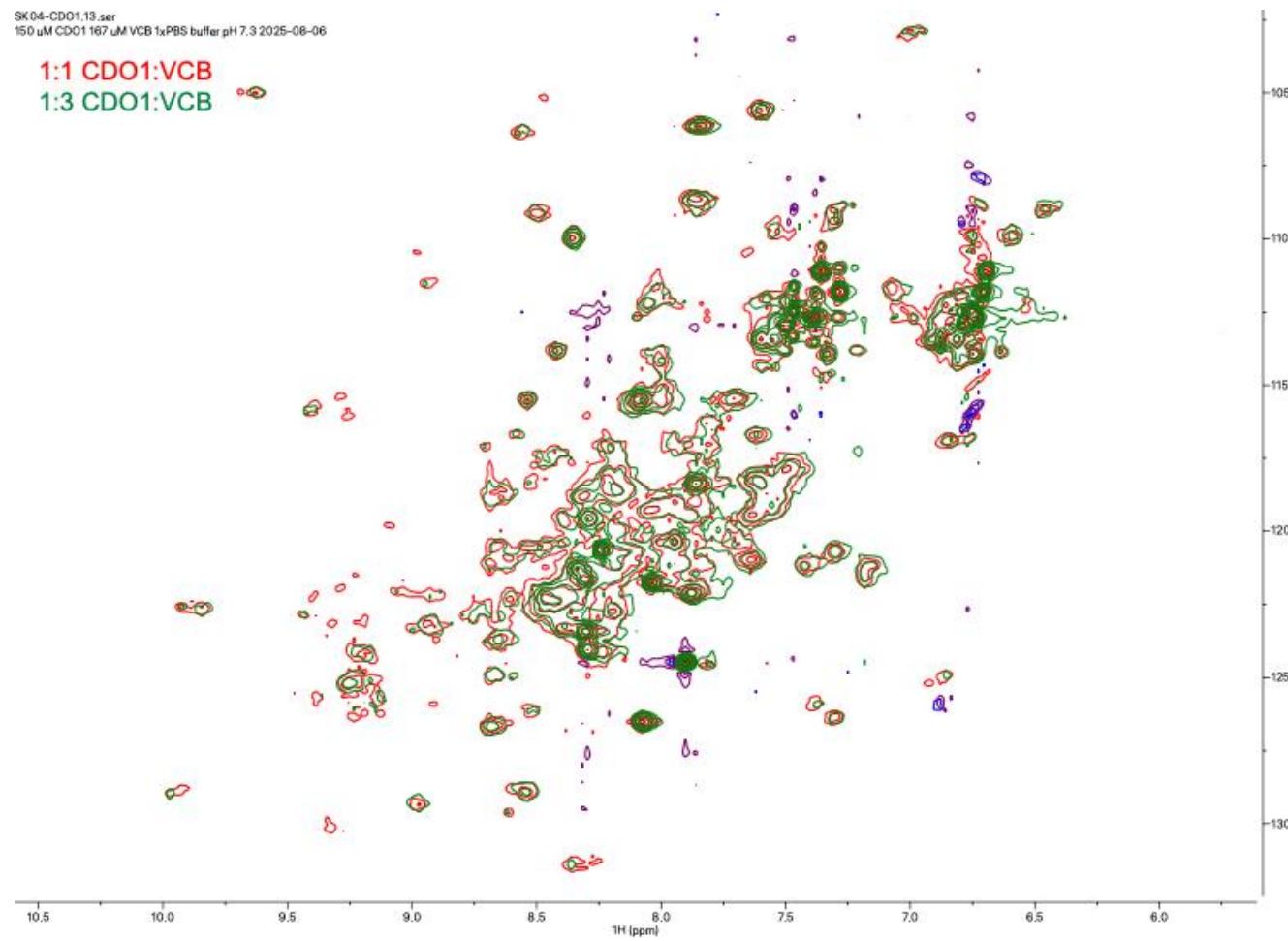


Figure 62: $^1\text{H}/^{15}\text{N}$ -HSQC of CDO1 with and without 2.8-fold excess of VCB.

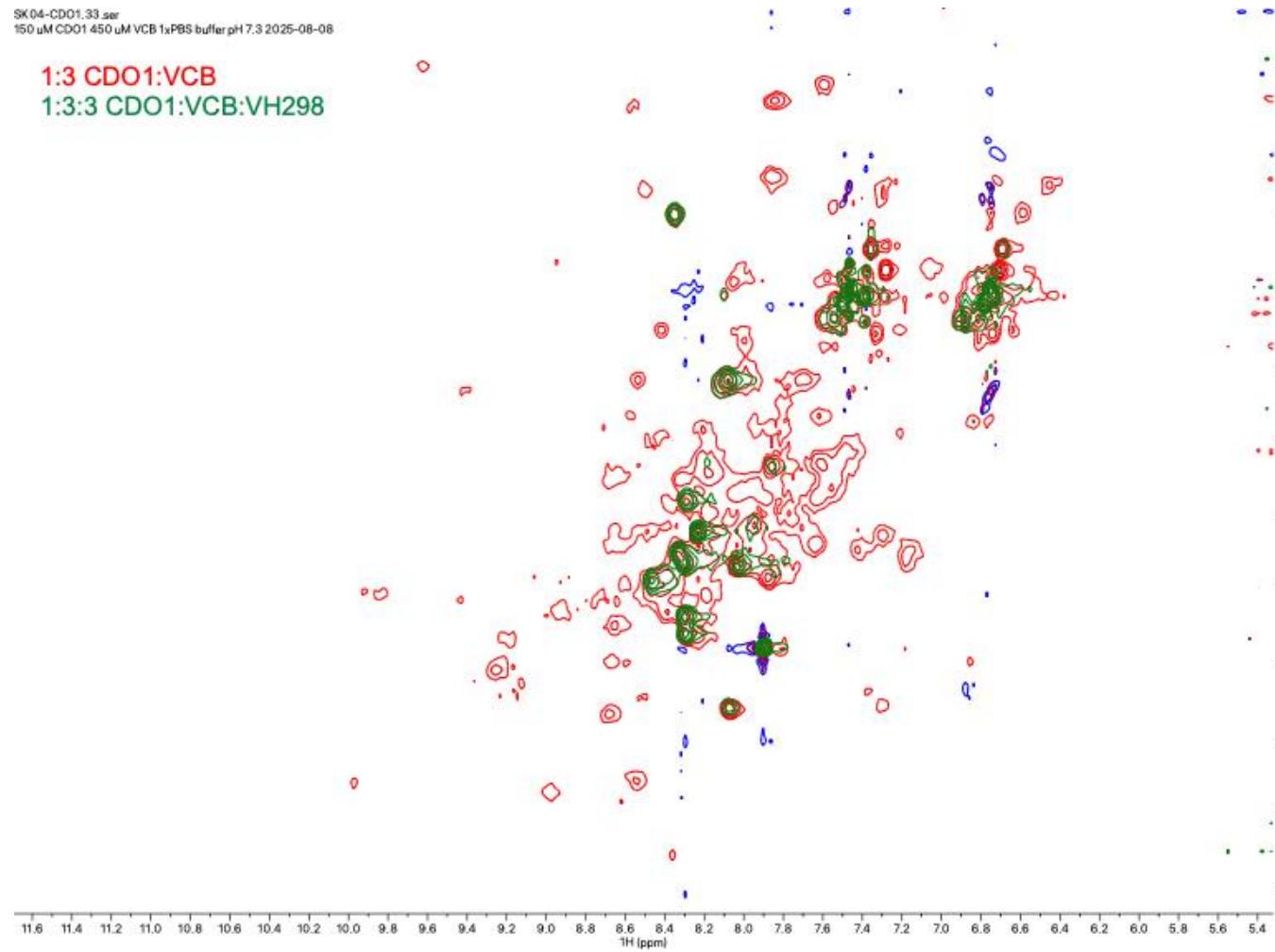
Kevin: Interaction observed between CDO1 and labelled VCB in 1:2.8 ratio

1:1 CDO1:VCB vs. 1:3 CDO1:VCB

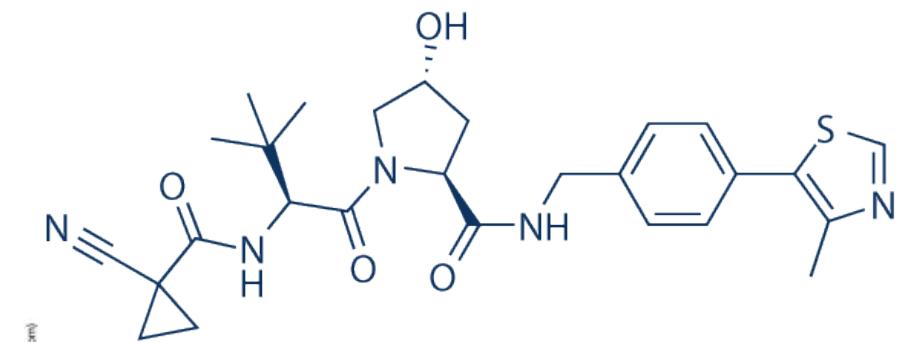


SK04-CDO1.33.ser
150 uM CDO1 450 uM VCB 1xPBS buffer pH 7.3 2025-08-08

1:3 CDO1:VCB
1:3:3 CDO1:VCB:VH298

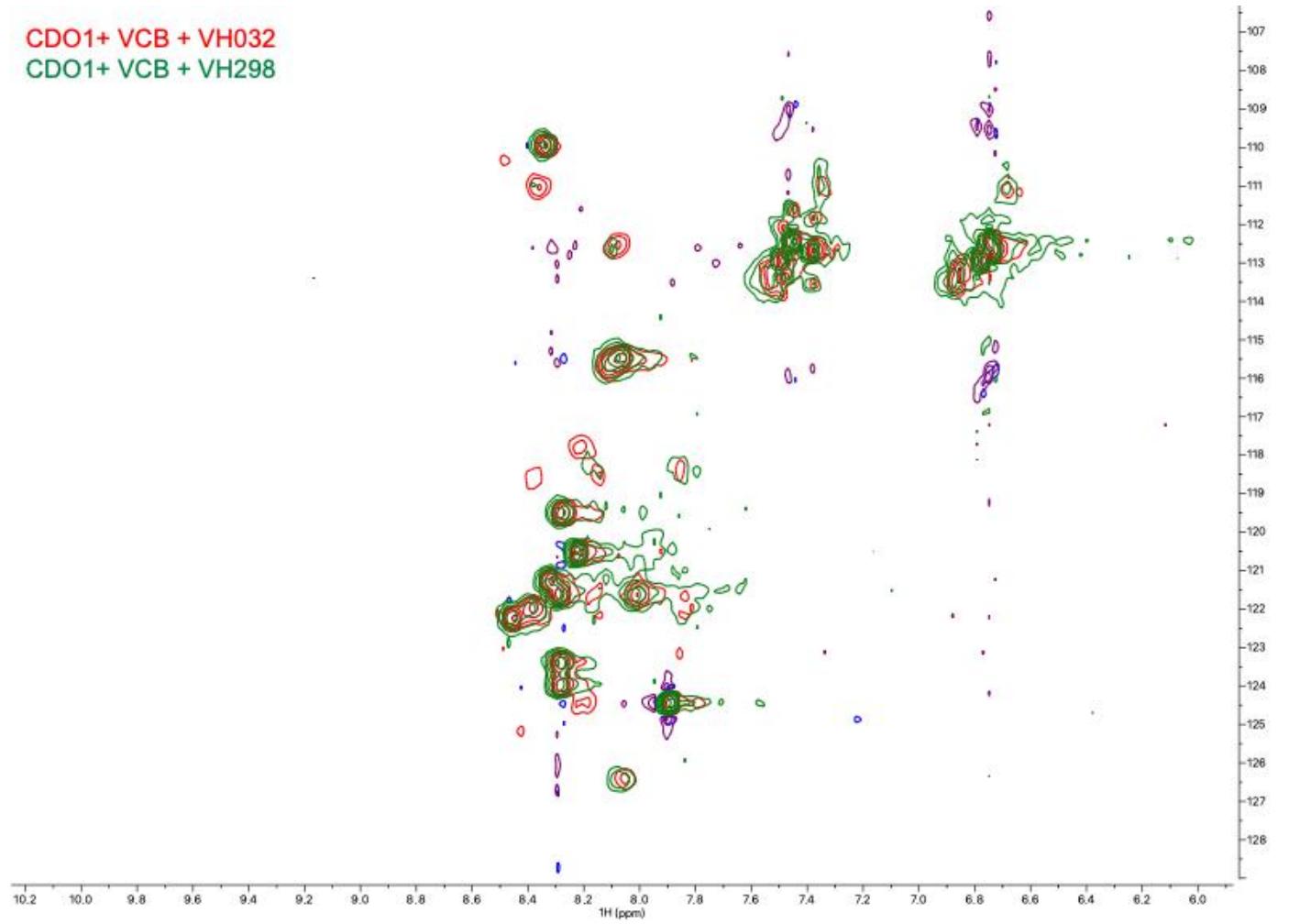


1:3 CDO1:VCB vs 1:3:3 CDO1:VCB:VH298





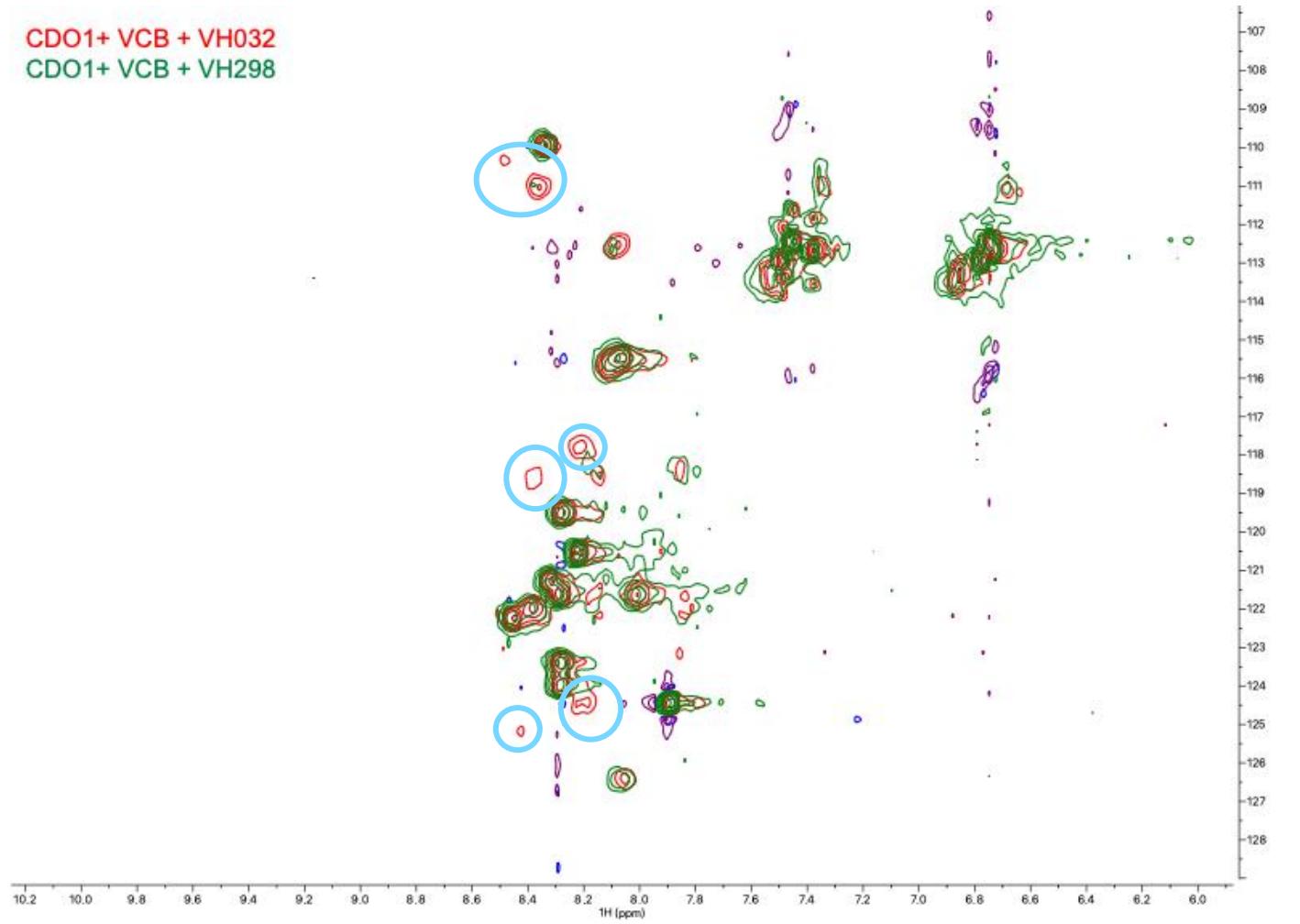
CDO1+ VCB + VH032
CDO1+ VCB + VH298



VH032 vs VH298



CDO1+ VCB + VH032
CDO1+ VCB + VH298



VH032 vs VH298



Conclusions

- No intrinsic interaction between VCB and CDO1 alone, VH298 required for glueing
 - Loss of signal is only detected when VH298 is present
- Next steps
 - NMR for CDO1 + VH298
 - Backbone resonance assignment
 - Obtain binding affinity; ITC, Helix, SPR
- Learning outcomes
 - ~87 mg VCB
 - ~13 mg CDO1
 - ~3 mg Labelled CDO1



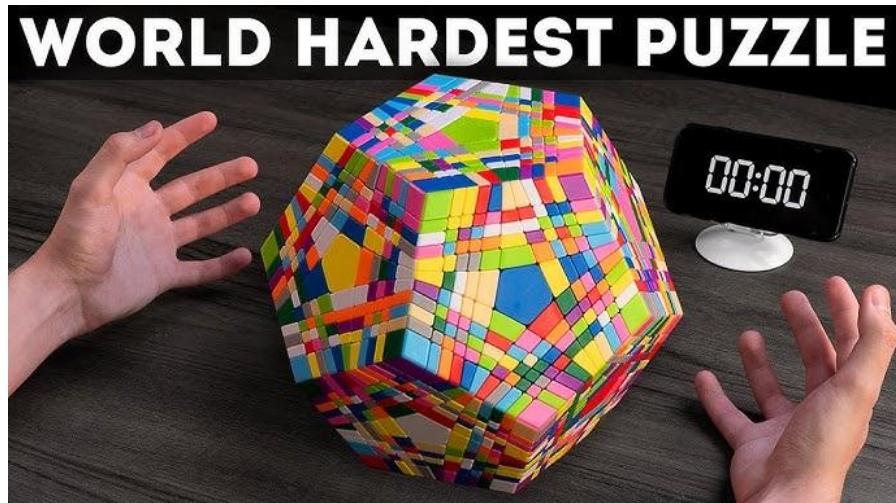
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Personal Experiences



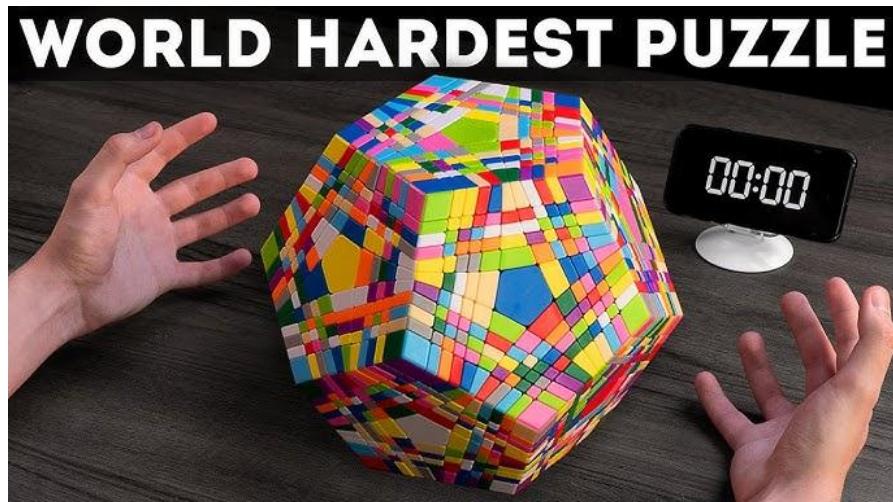
Personal Experiences



Week 1



Personal Experiences



Week 1



Week 5



Personal Experiences

Bacterial cell culture

ÄKTA HPLC system

Manual column

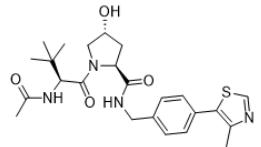
NMR



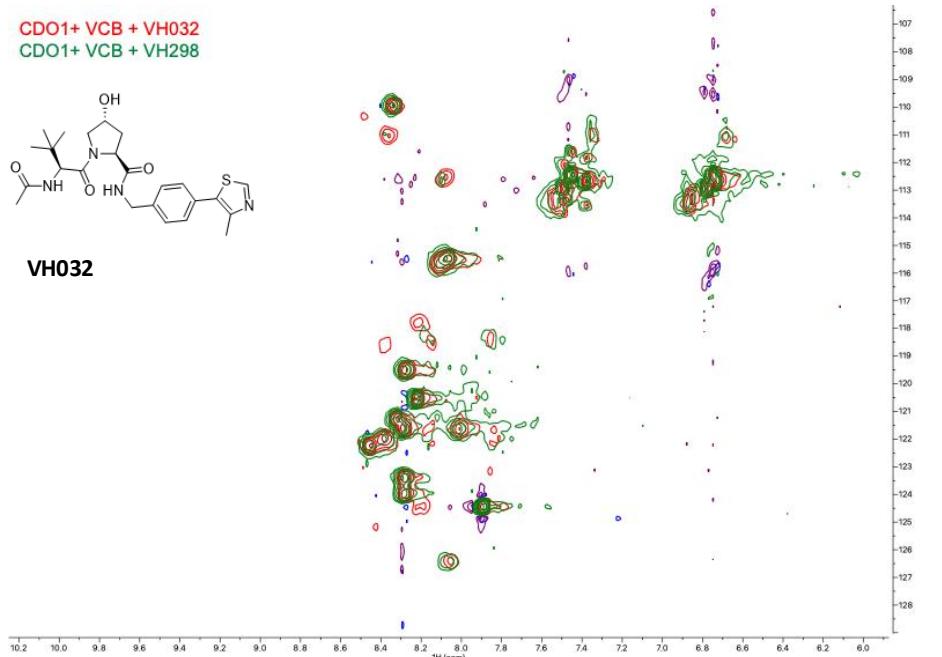
Personal Experiences



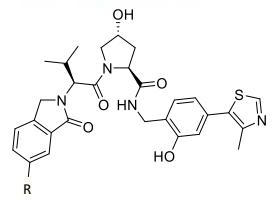
CDO1+ VCB + VH032
CDO1+ VCB + VH298



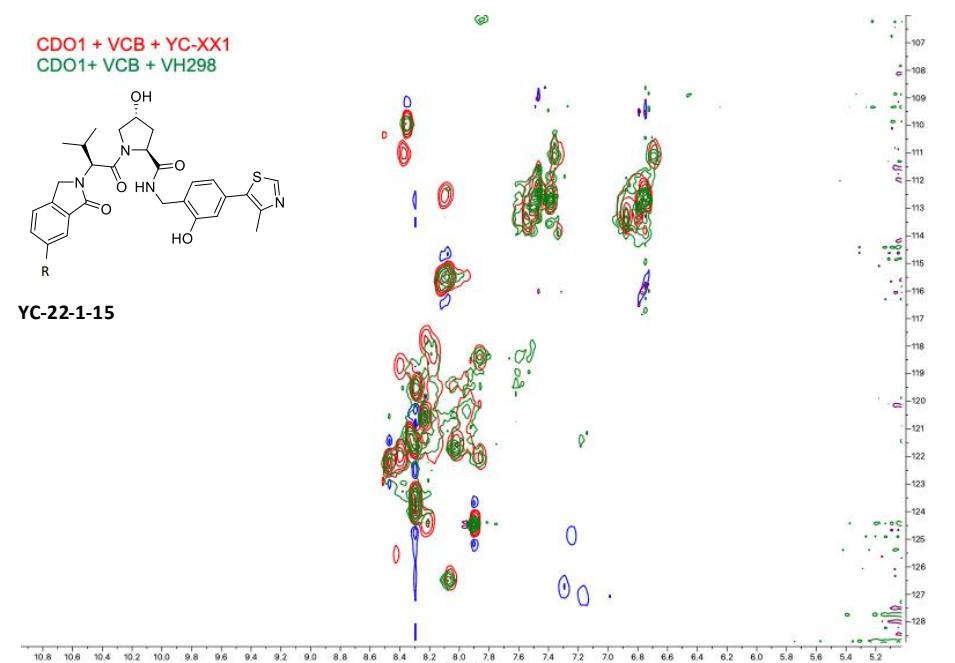
VH032



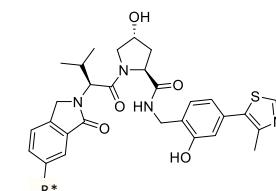
CDO1 + VCB + YC-XX1
CDO1+ VCB + VH298



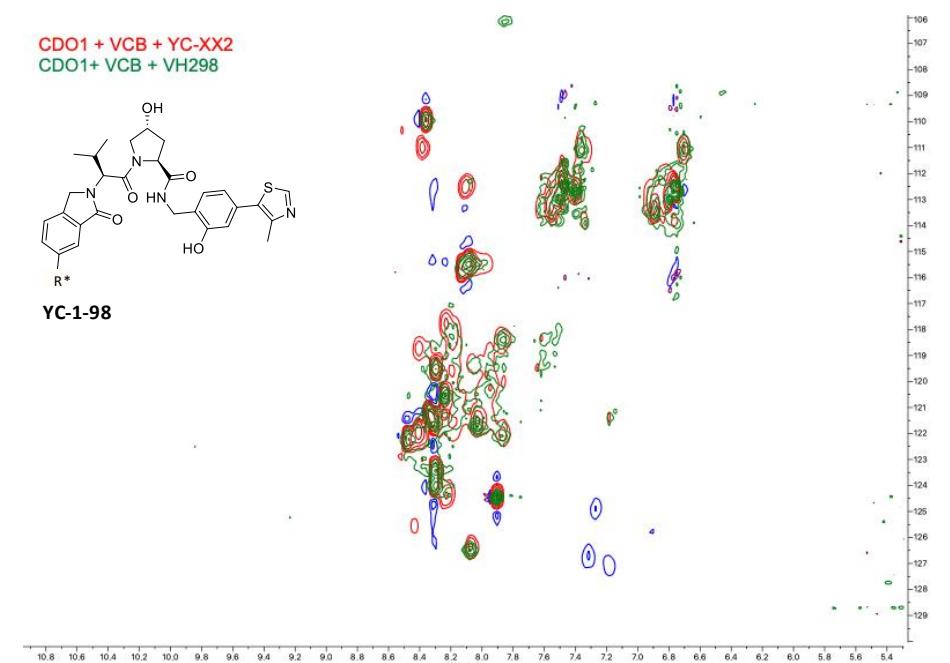
YC-22-1-15



CDO1 + VCB + YC-XX2
CDO1+ VCB + VH298



YC-1-98



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