



Centre for Targeted
Protein Degradation
University of Dundee

innovate
collaborate
inspire

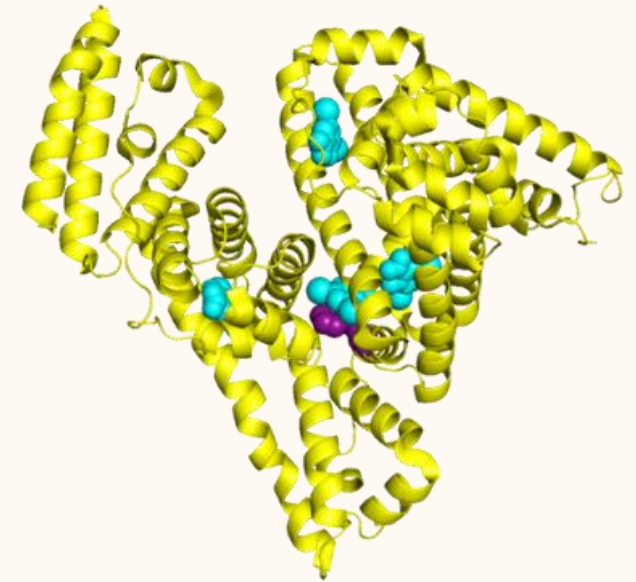
Does VH298 glue CDO1 to VHL?

My 6-week summer project at the CeTPD

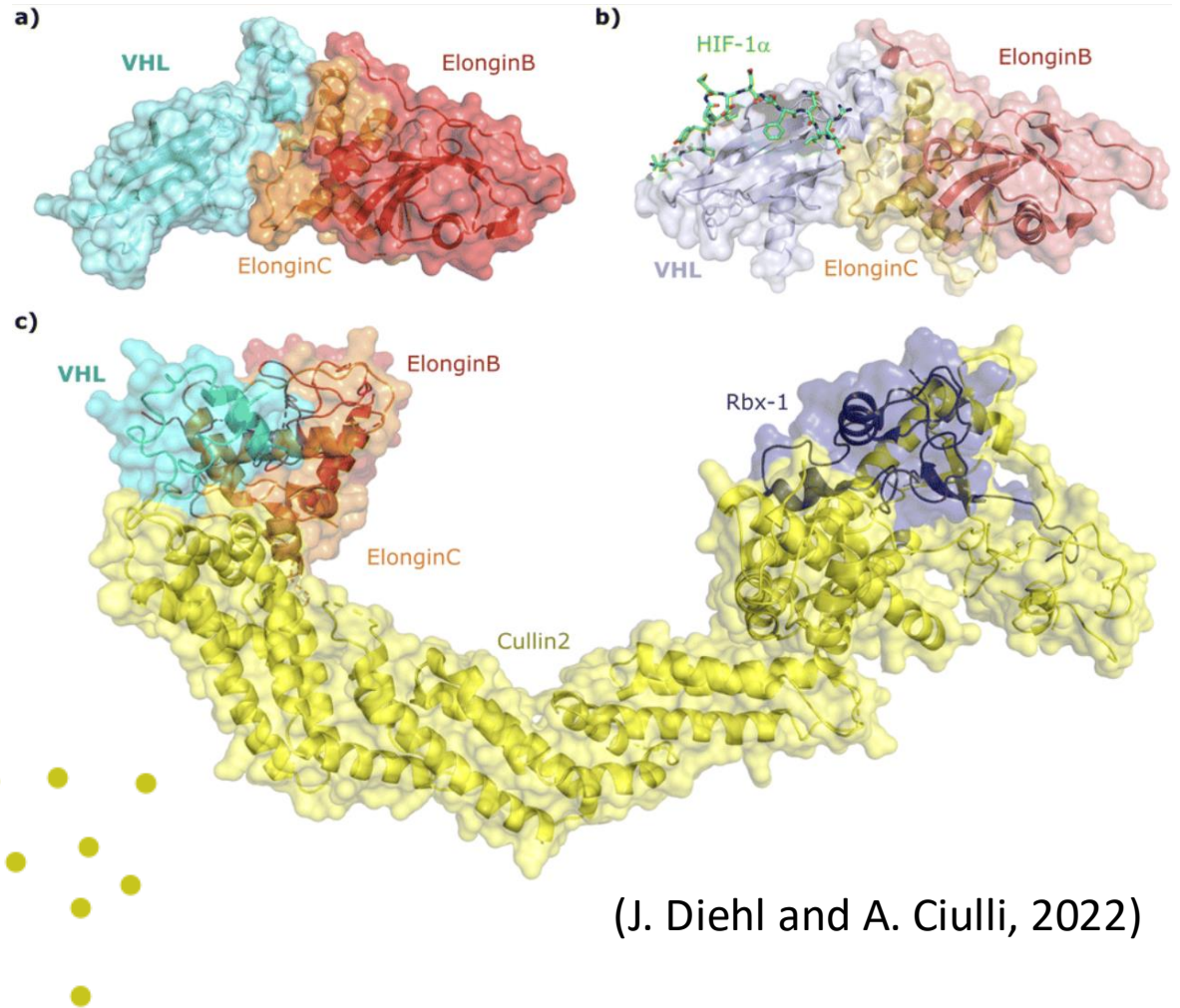
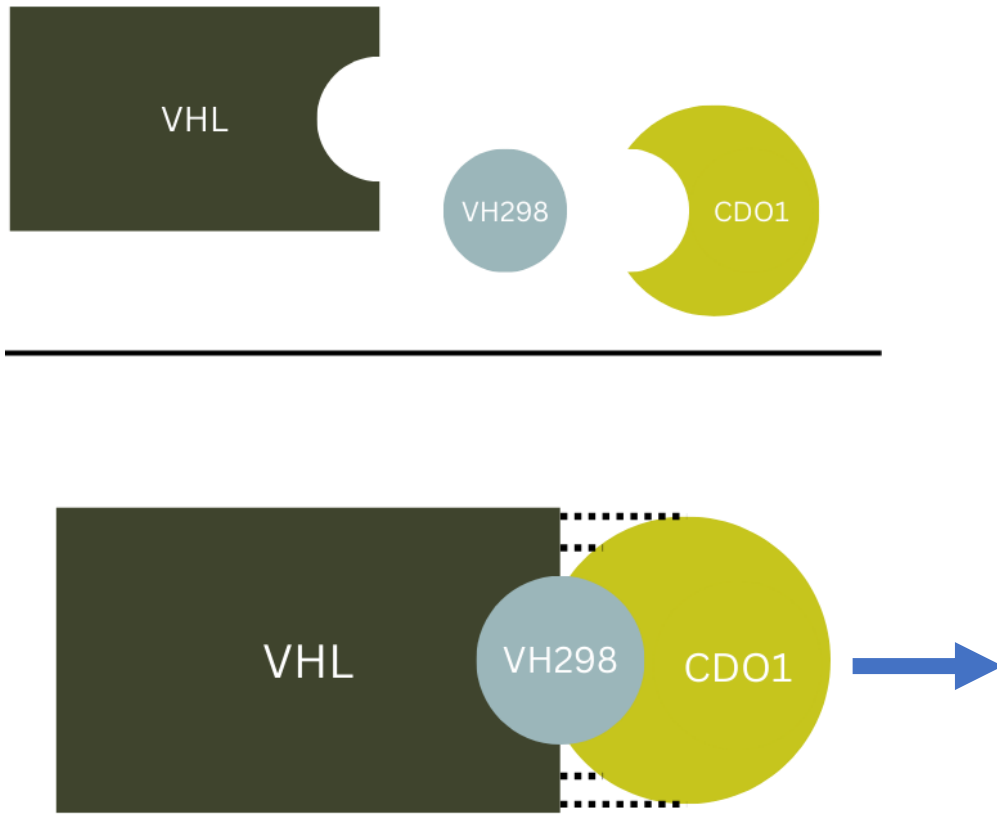
Sansara Klinsukont; 2025 Summer Student



A little about me



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

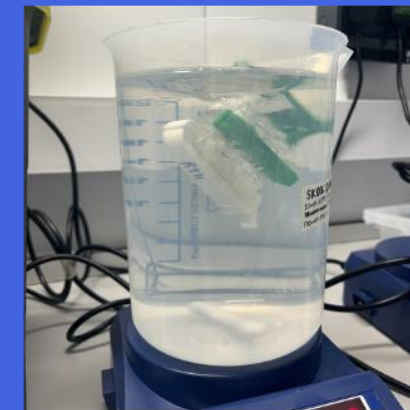
Express and purify VCB

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.

VCB His-Trap and Reverse His-Trap



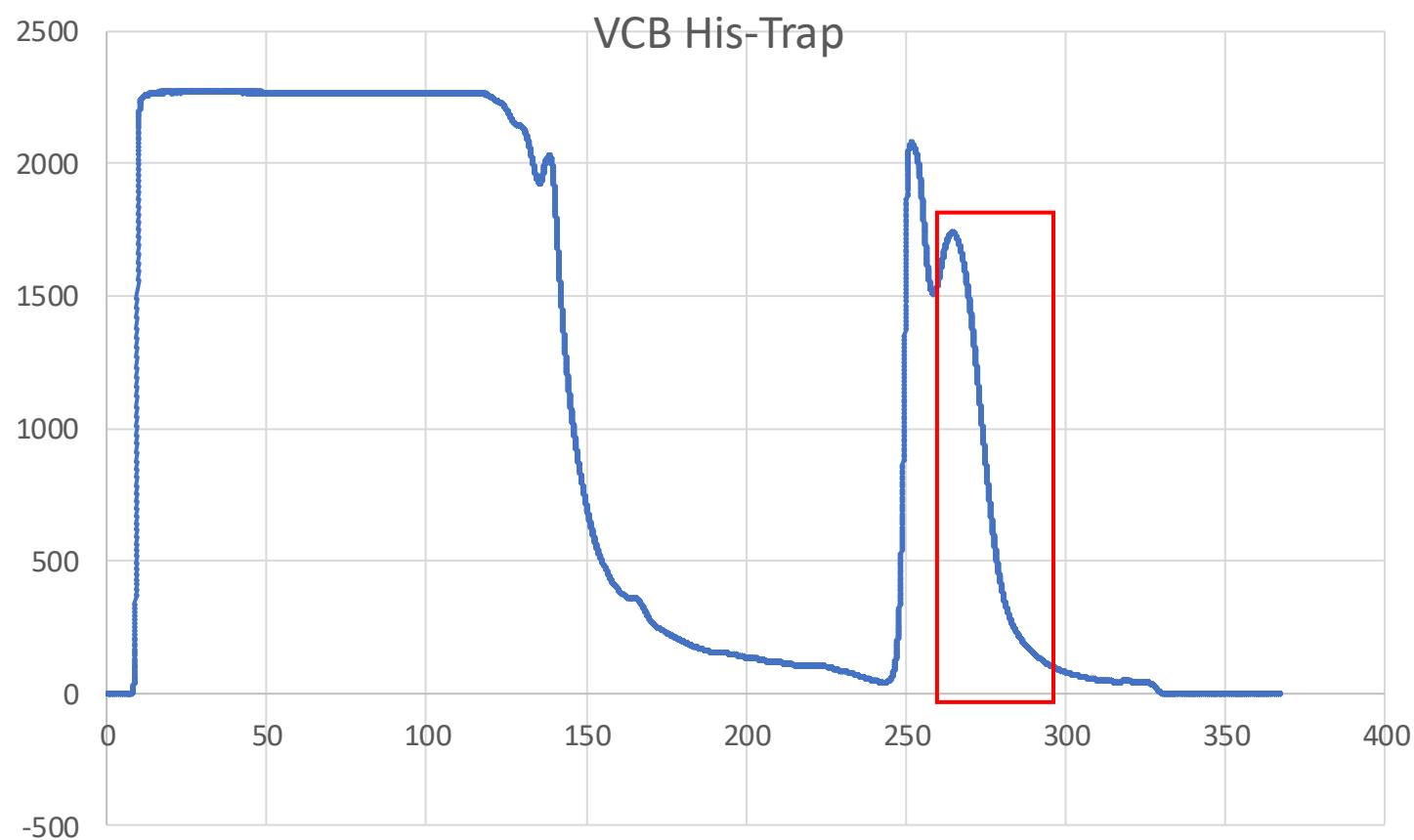
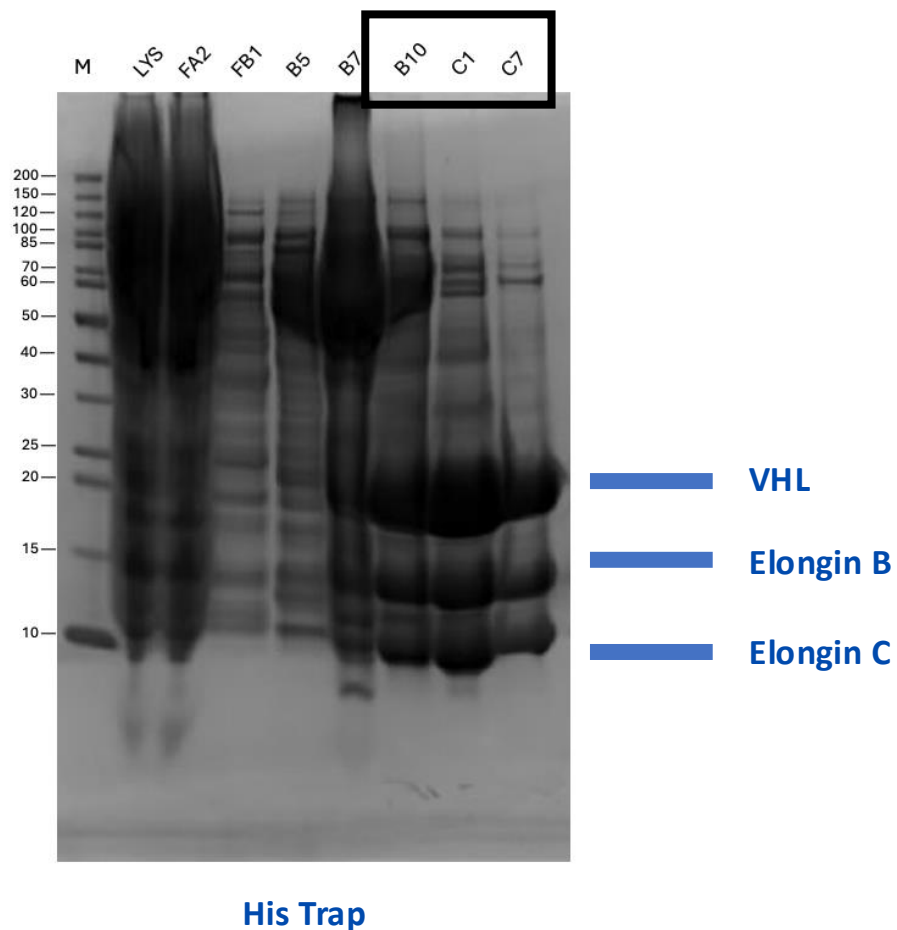
VCB His-Trap and Reverse His-Trap





Workflow

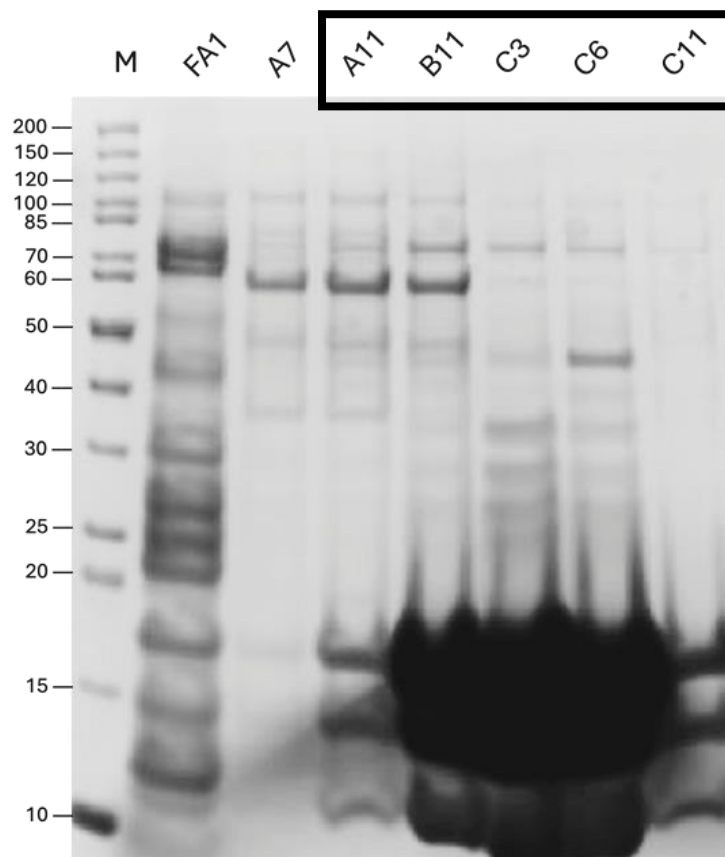
VCB His-Trap and Reverse His-Trap



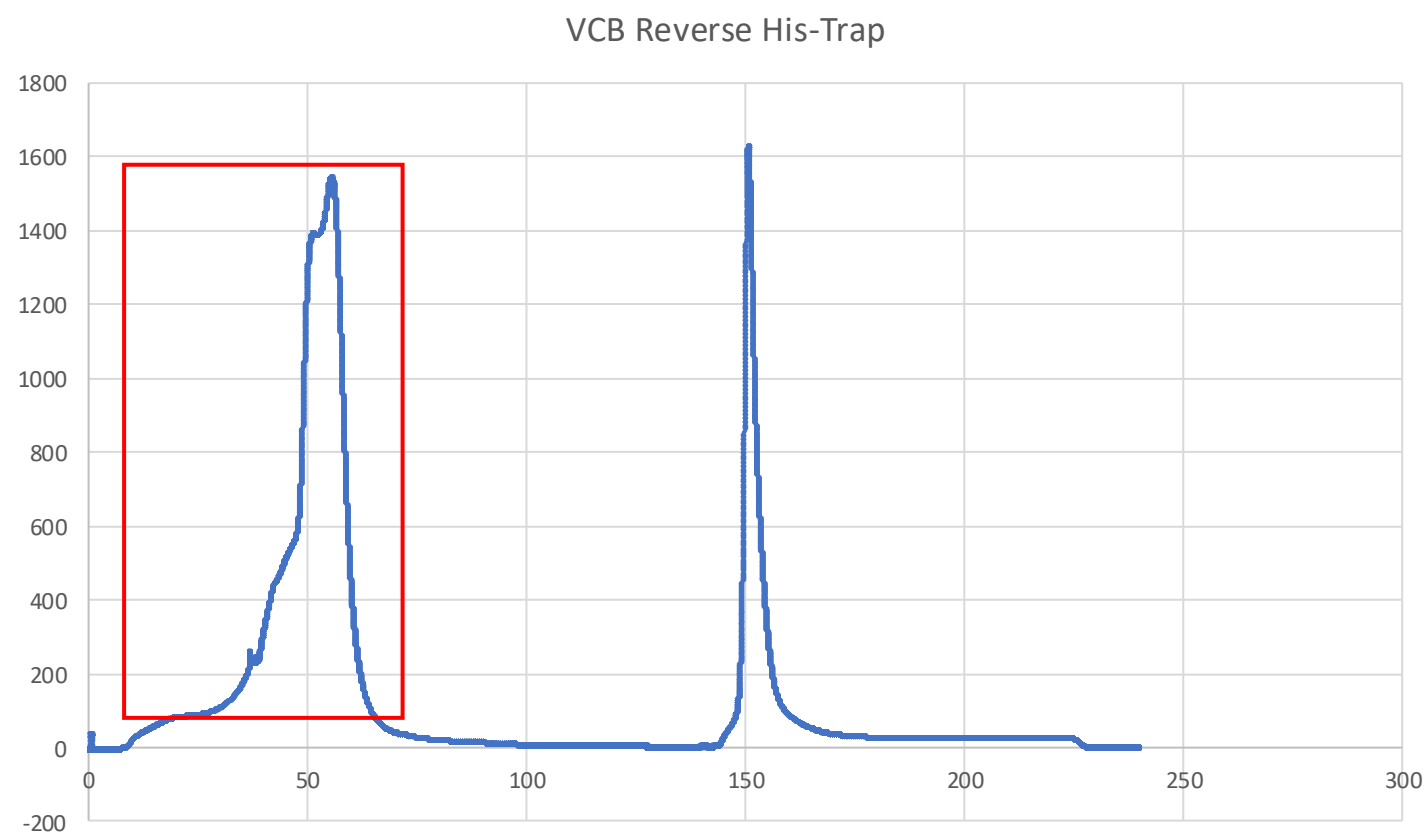


Workflow

VCB His-Trap and Reverse His-Trap

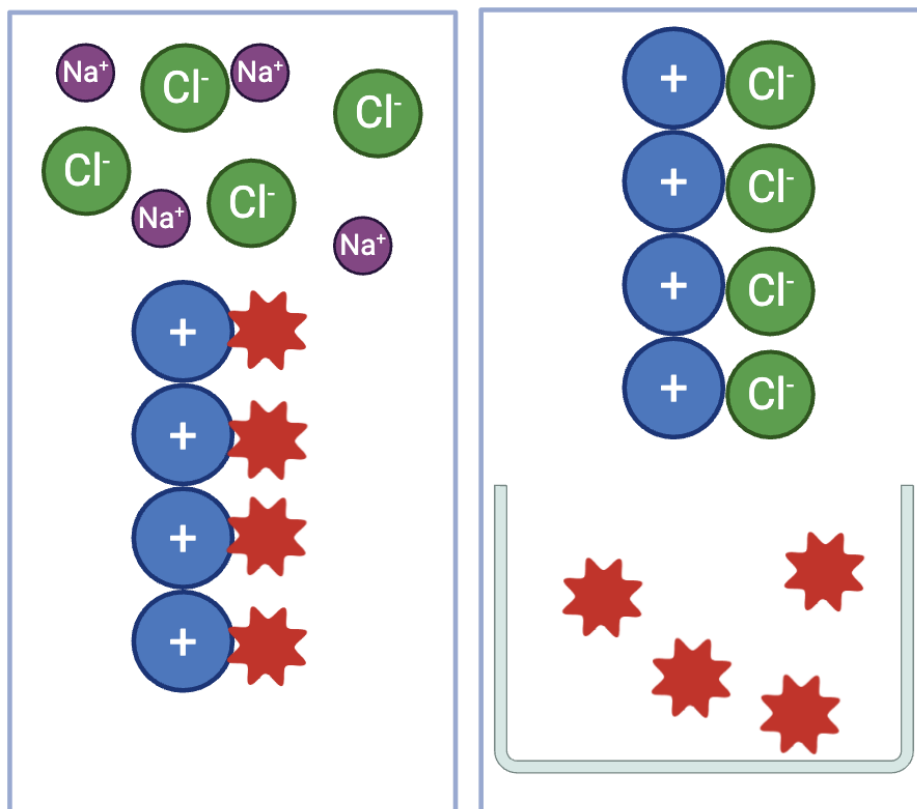


Reverse His-Trap



Workflow

VCB Anion-Exchange Chromatography (AEC)



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

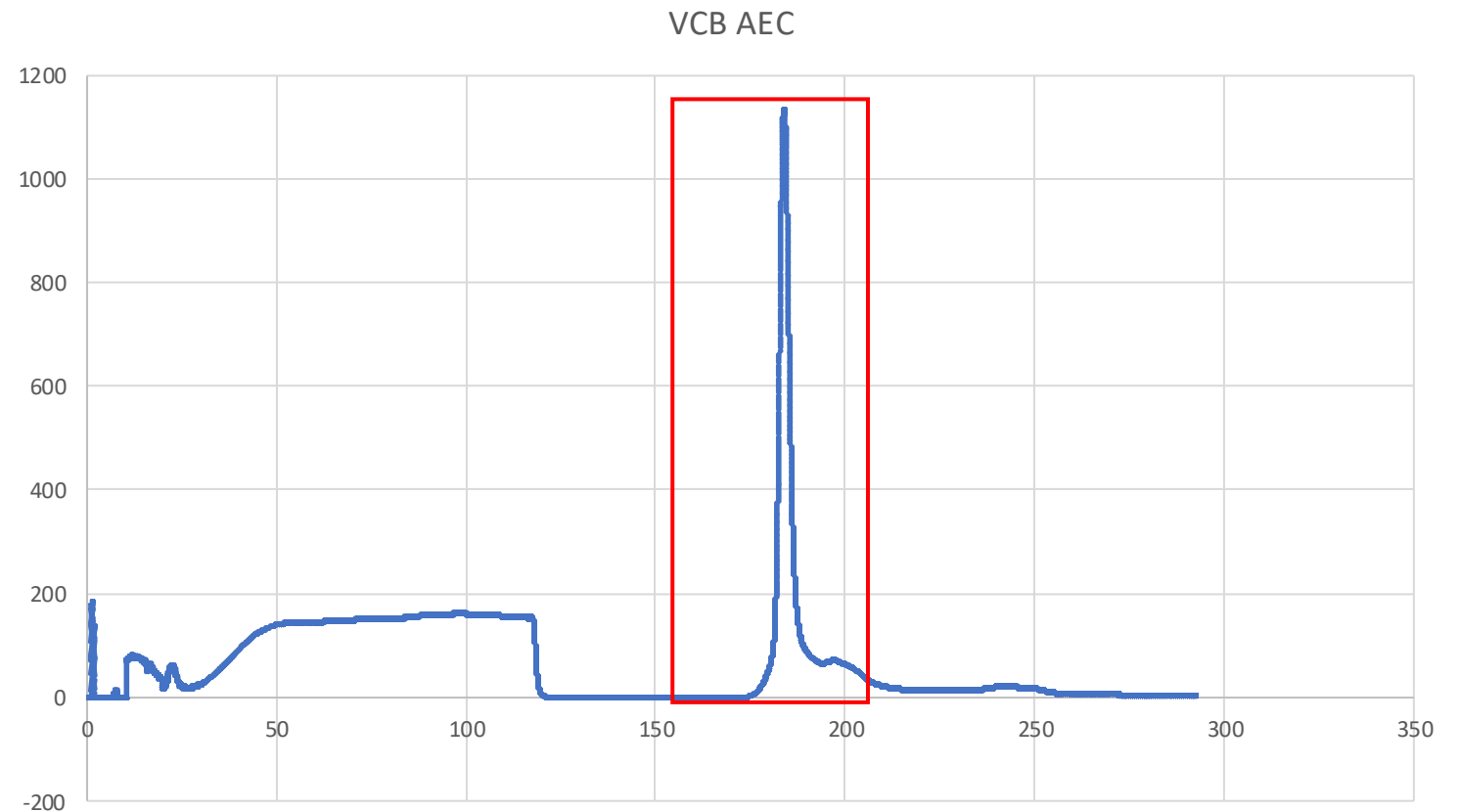
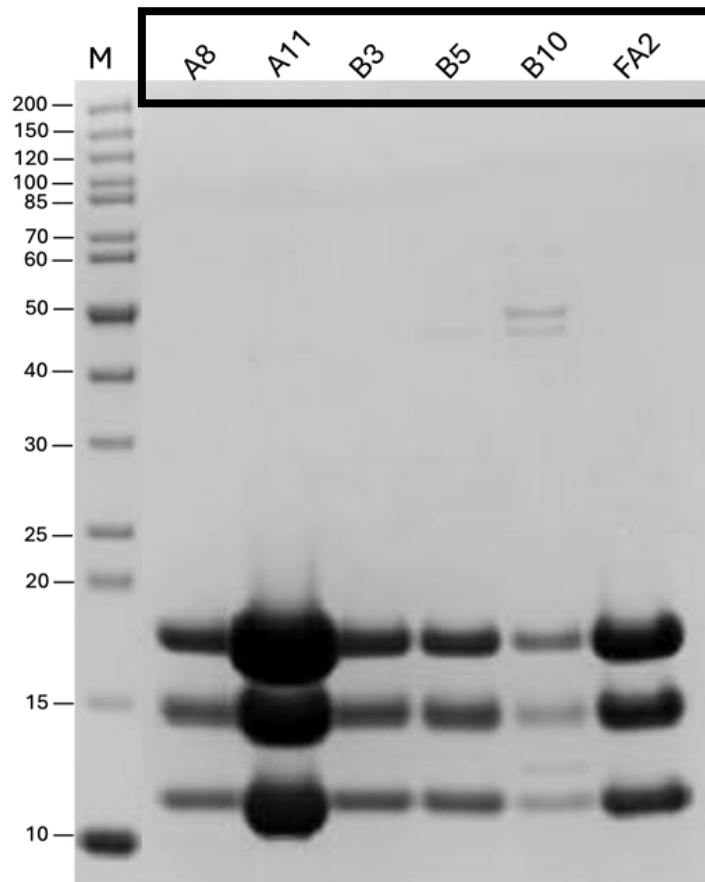
Elute with high conc. NaCl, Cl^- displaces bound VCB

VCB washes off.



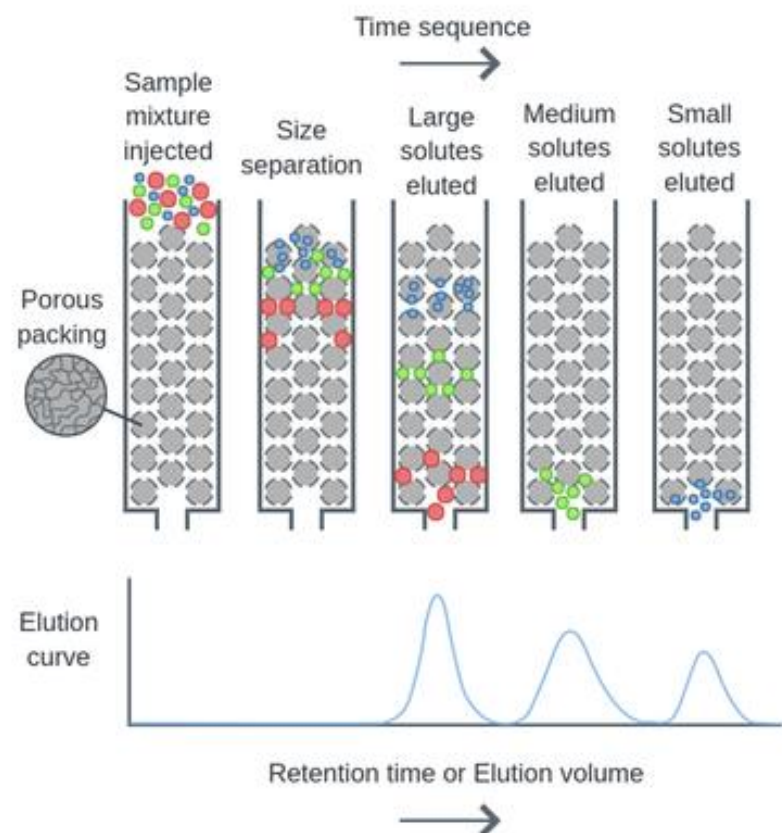
Workflow

VCB Anion-Exchange Chromatography (AEC)



Workflow

VCB Size-Exclusion Chromatography (SEC)



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

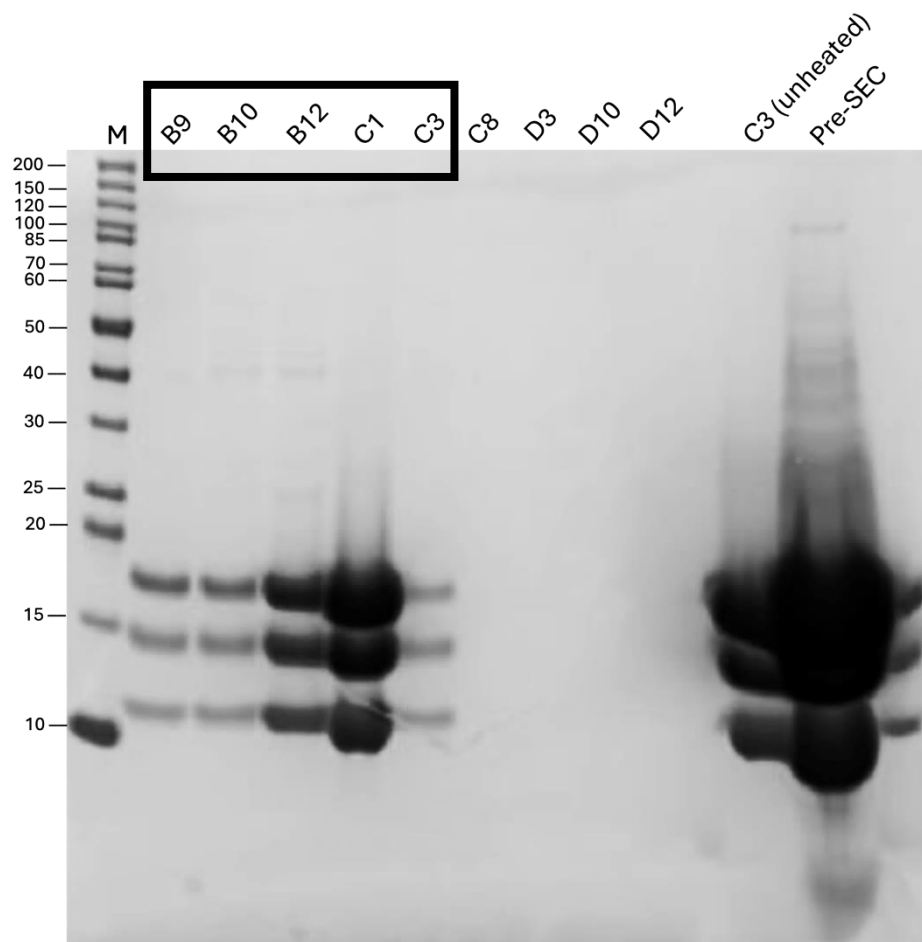
VCB eventually flows through the column and is collected

(Labster Theory pages)



Workflow

VCB Size-Exclusion Chromatography (SEC)



VCB concentration

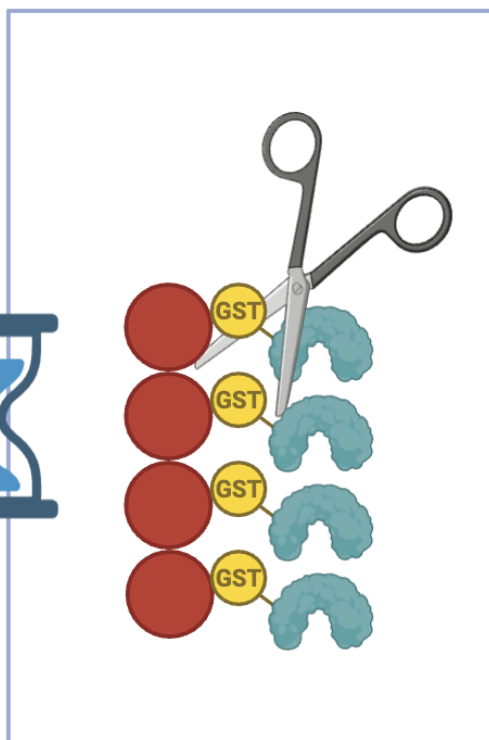
- 52.6 mg/mL
- 1.27 mM
- ~400 μ L total

Workflow

CDO1 GST-Trap (Gravity column)

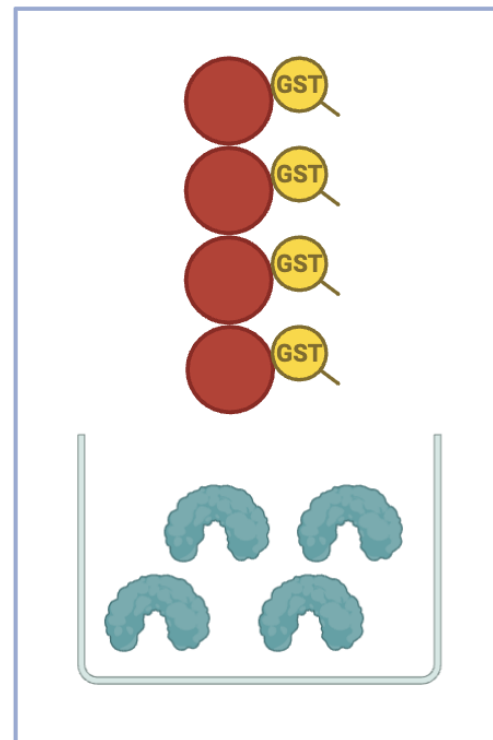


GST-Tags bind to
Glutathione beads.



Allow binding, wash off
unbound protein.

Cleave tag overnight
(precision protease).

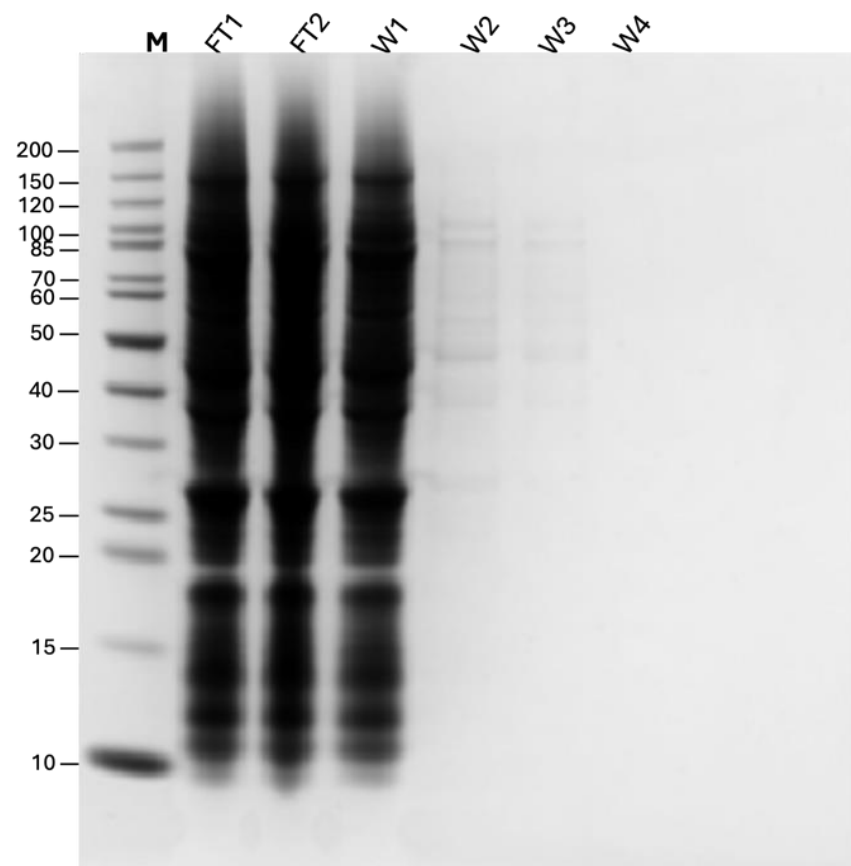


Wash column to retrieve
CDO1.

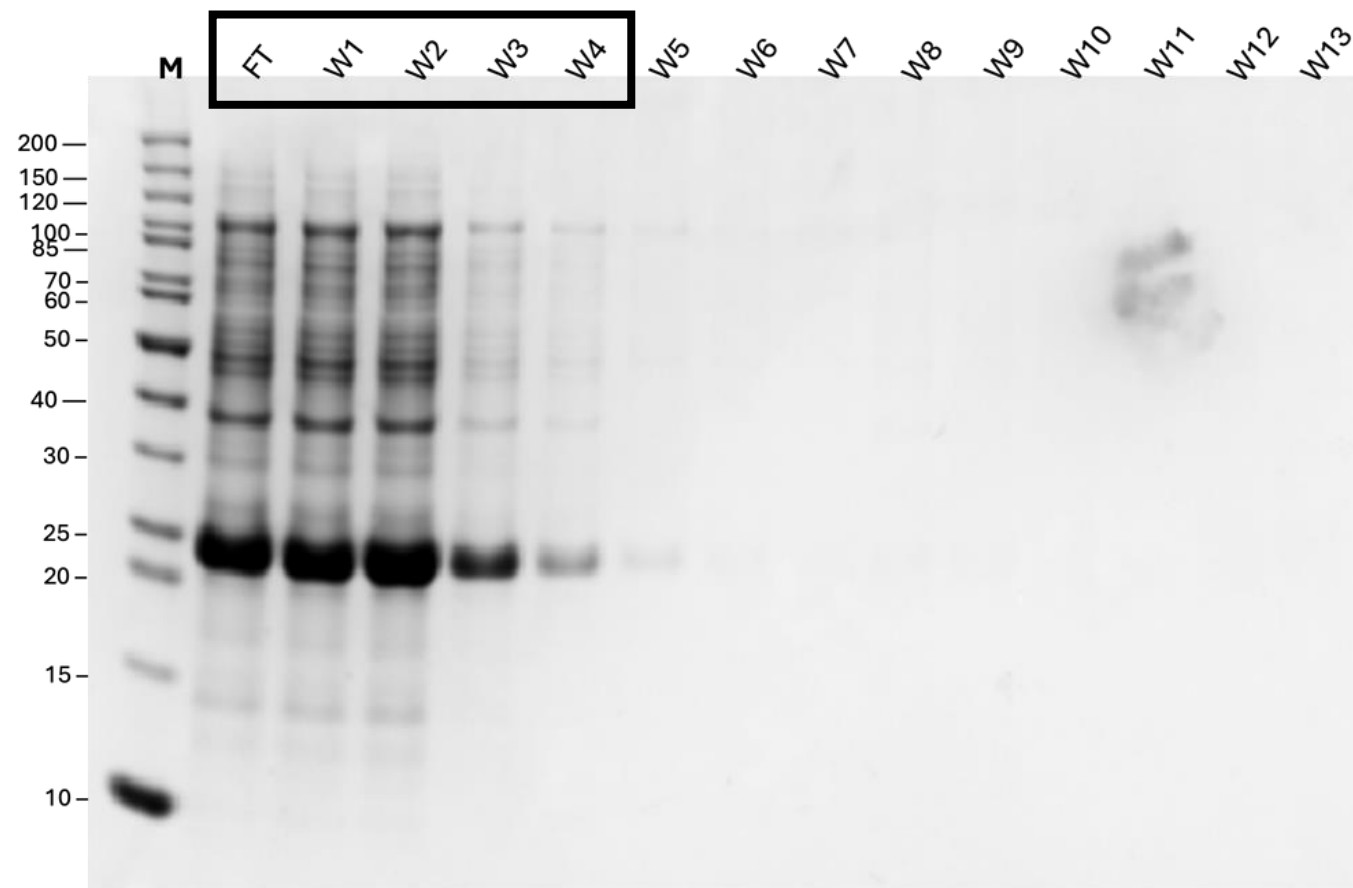


Workflow

CDO1 GST-Trap

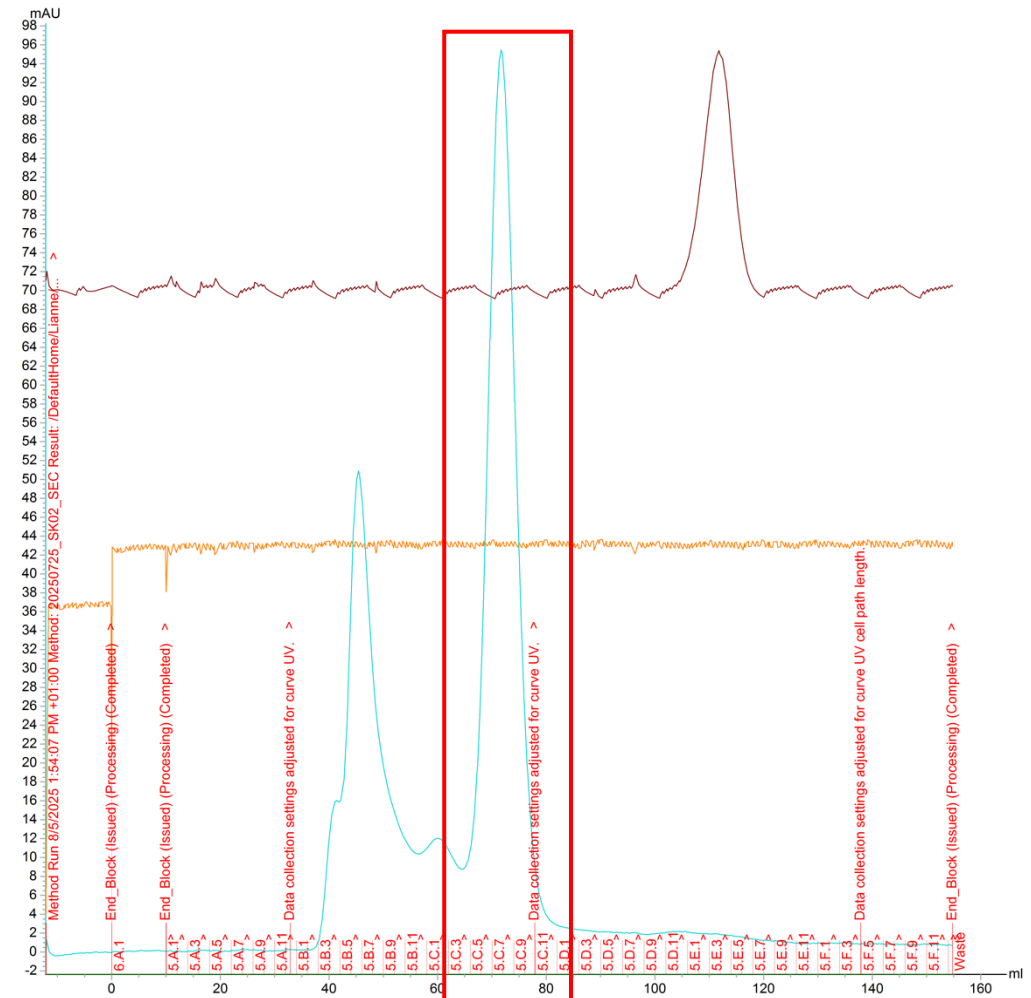
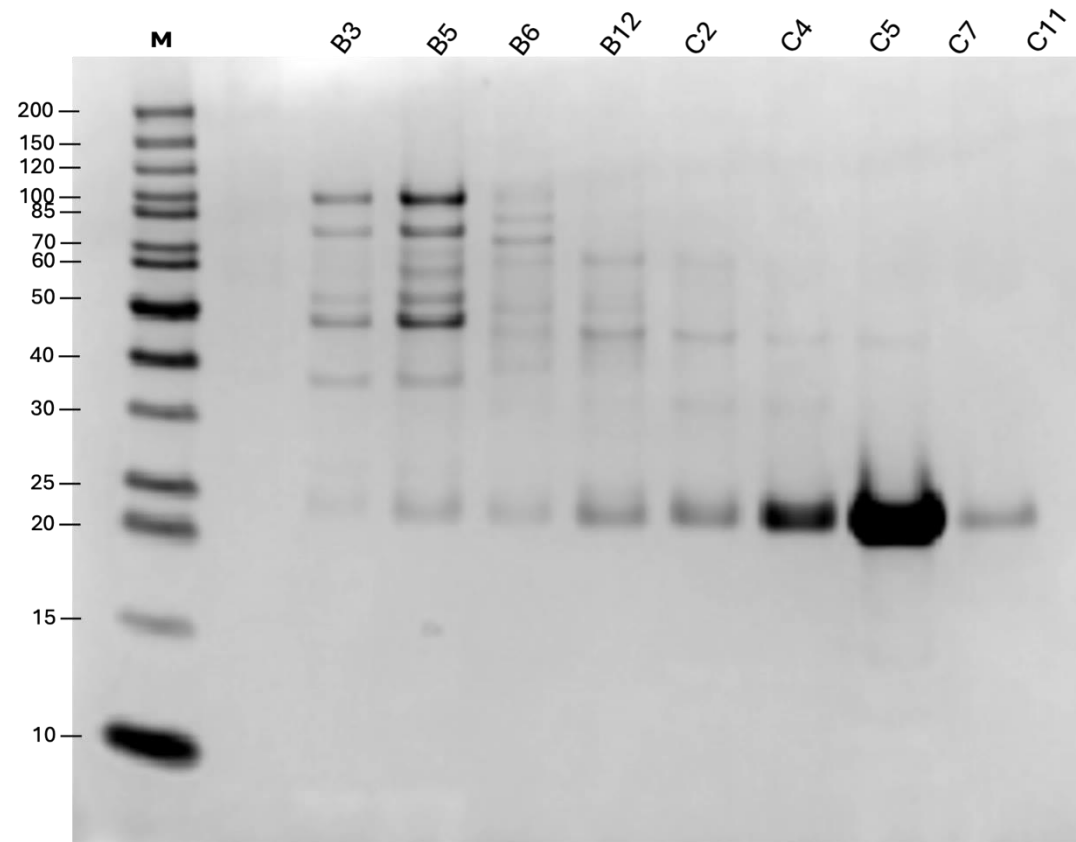


GST-Trap: Initial wash



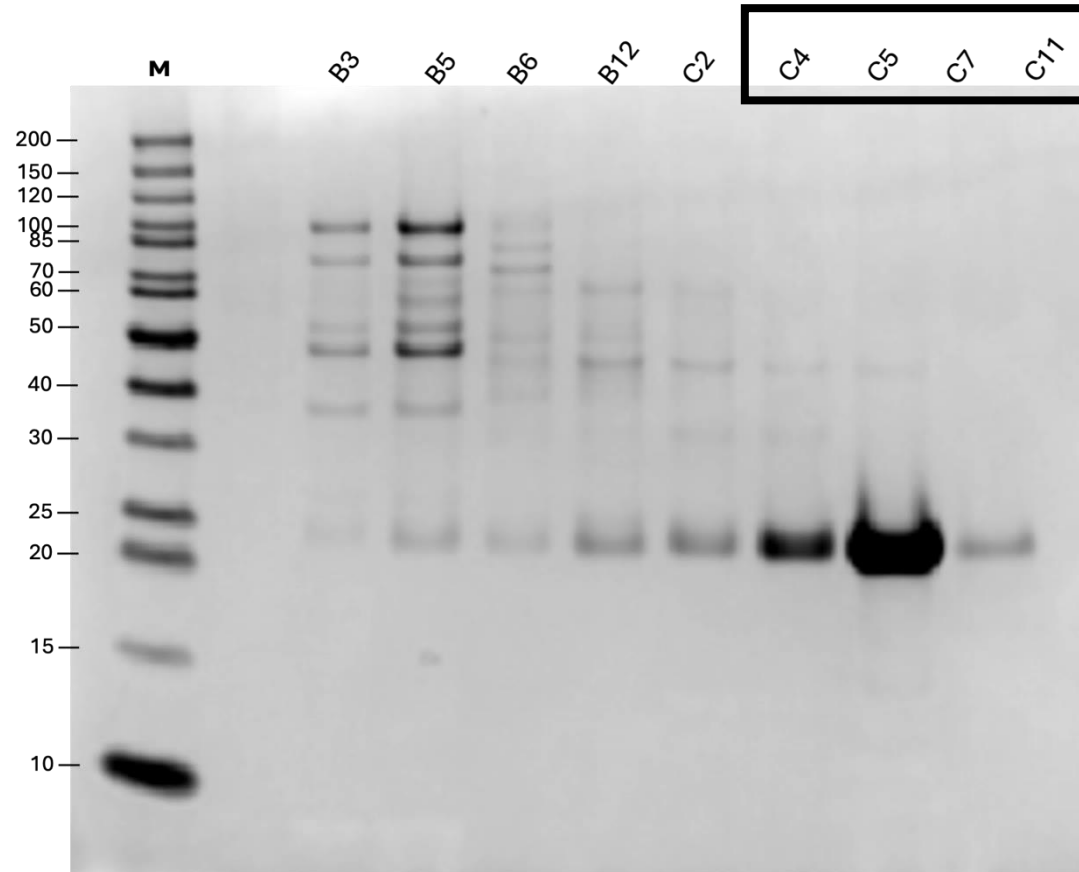
GST-Trap: Washes after binding and cleaving

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

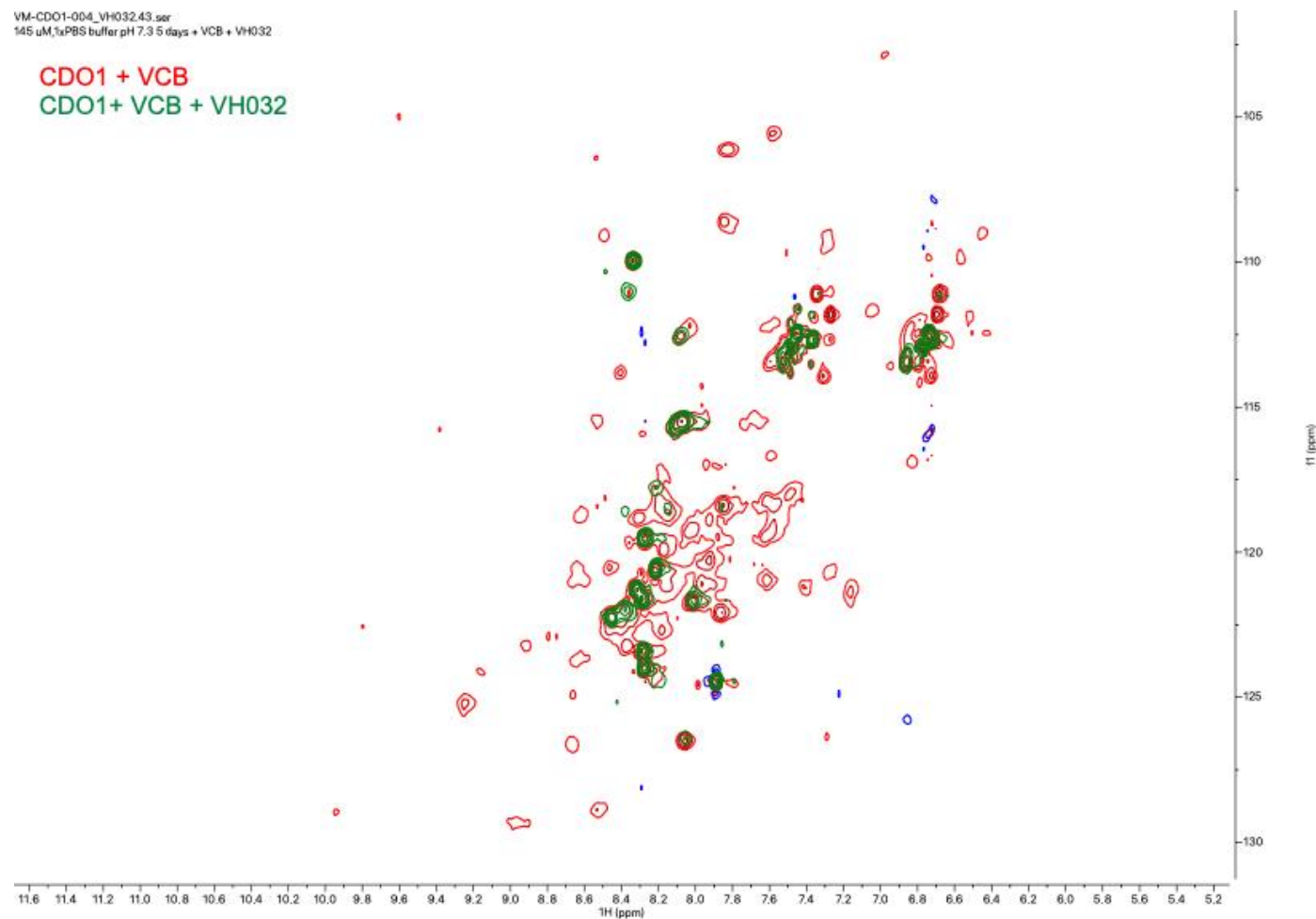
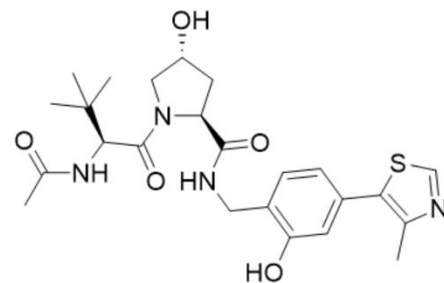
- 8.74 mg/mL
- 0.37 mM
- ~1500 μL total



VH032

VM-CDO1-004_VH032.43.ser
145 μ M, 1xPBS buffer pH 7.3 5 days + VCB + VH032

CDO1 + VCB
CDO1 + VCB + VH032

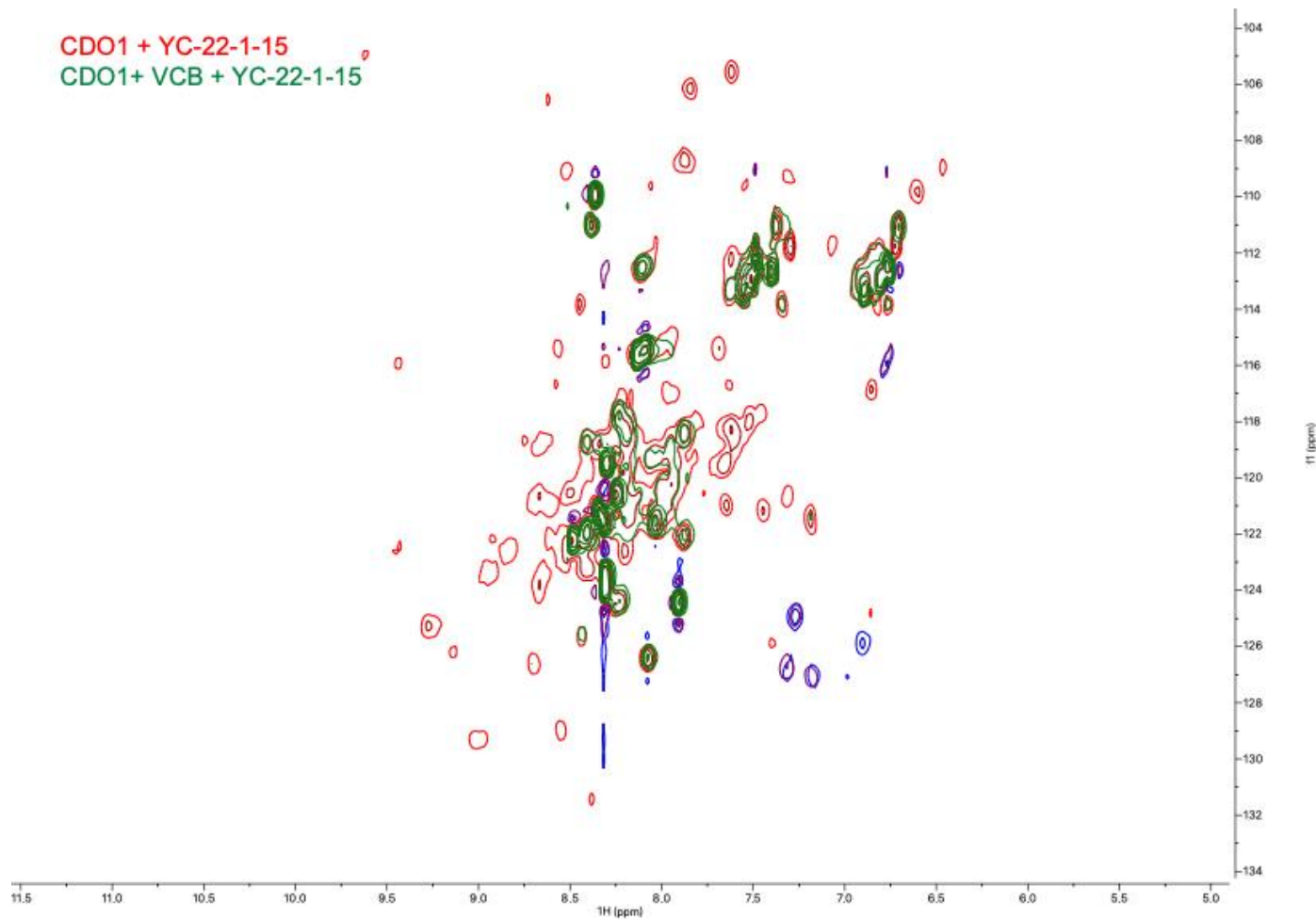
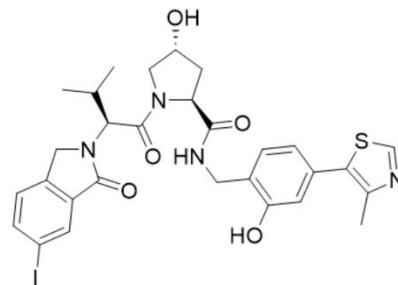


CDO1 + VCB vs. CDO1 + VCB + VH032



YC-22-1-15

CDO1 + YC-22-1-15
CDO1 + VCB + YC-22-1-15



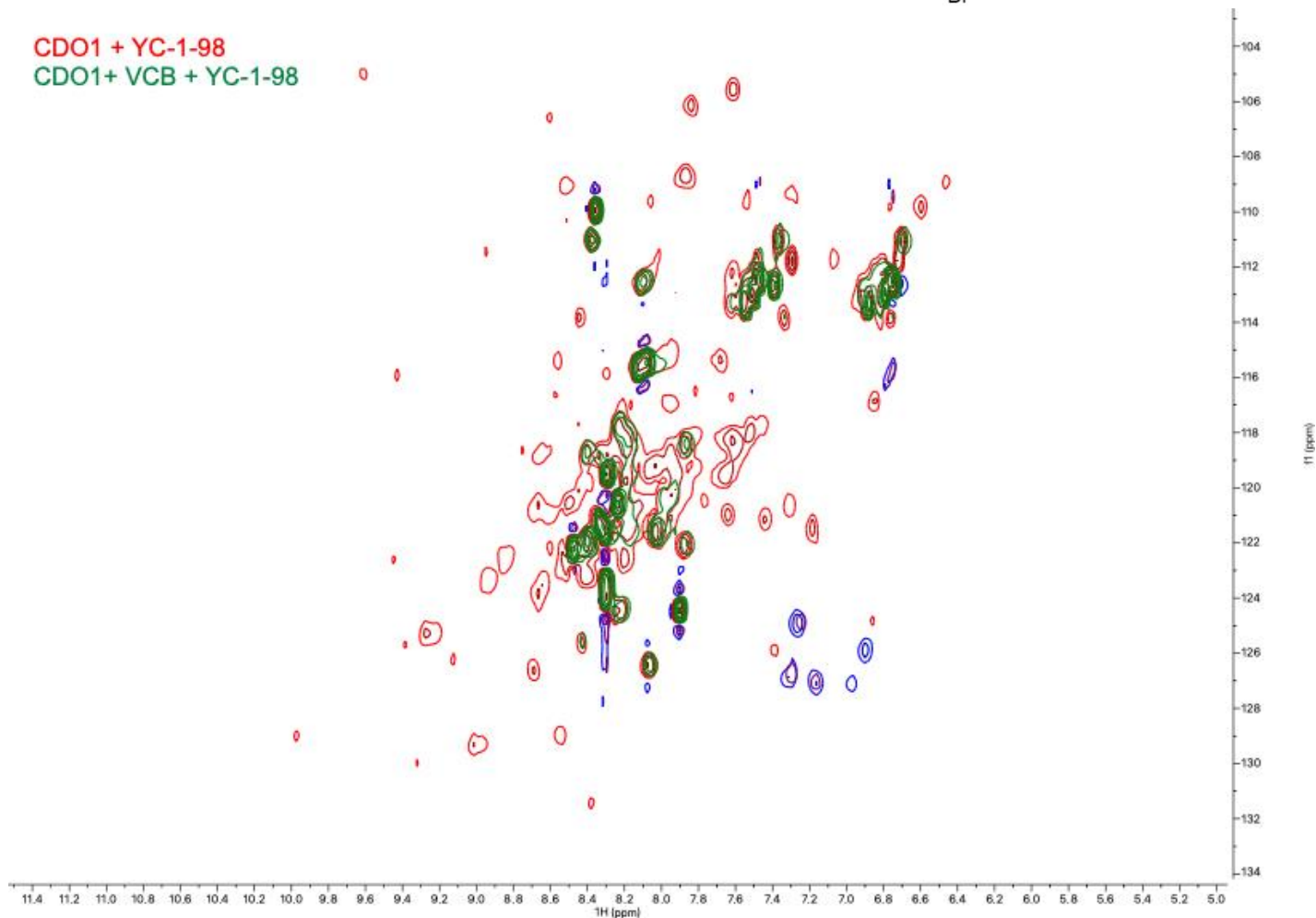
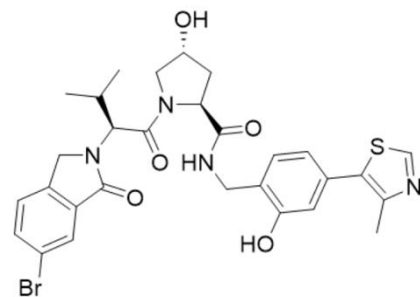
CDO1 + YC-22-15 vs. CDO1 + VCB + YC-22-15



YC-1-98

CDO1 + YC-1-98

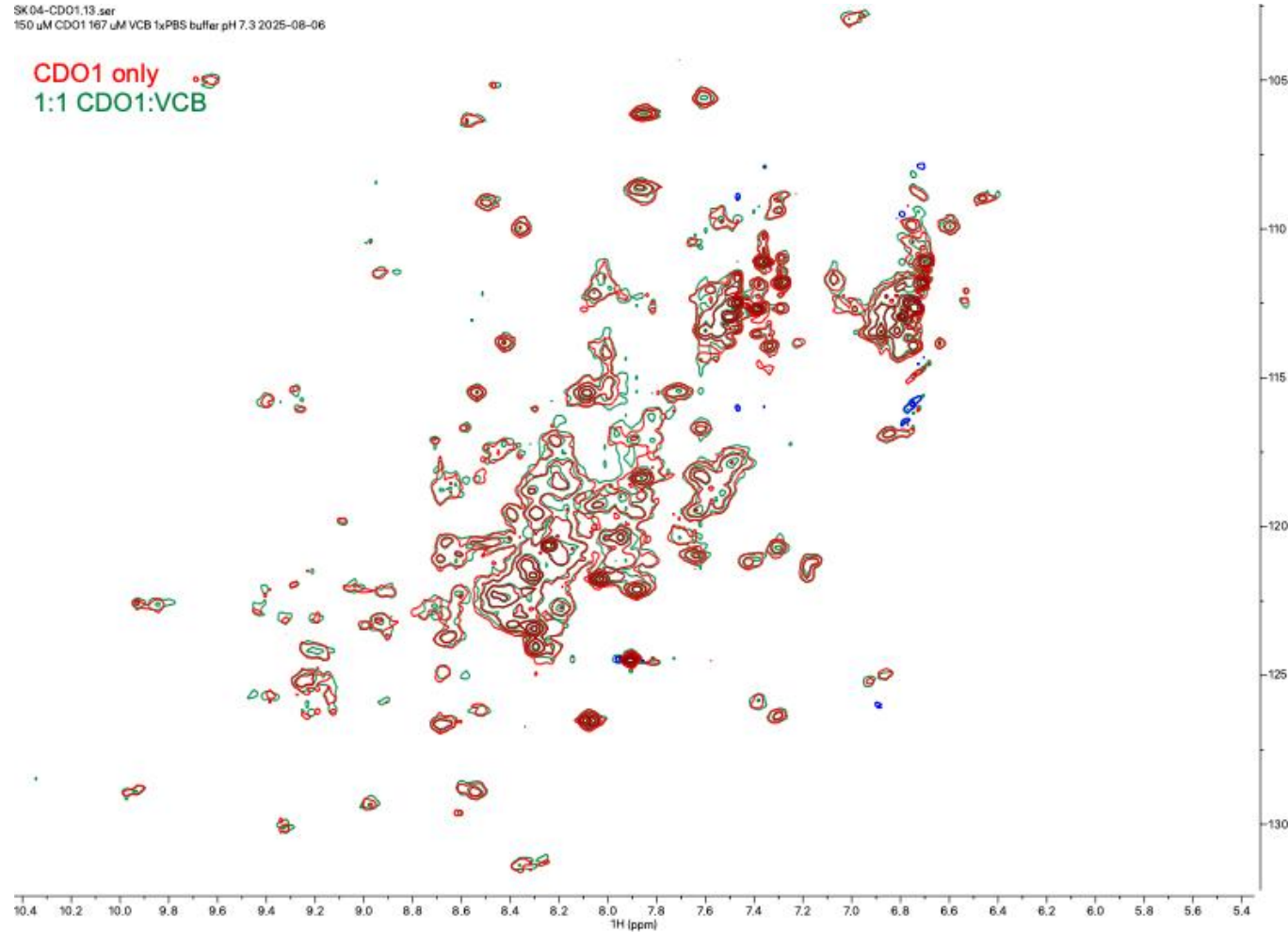
CDO1 + VCB + YC-1-98



CDO1 only vs. CDO1 + VCB + VH032



SK 04-CD01.13.ser
150 μ M CD01 167 μ M VCB 1xPBS buffer pH 7.3 2025-08-06



CD01 only vs. 1:1 CD01:VCB

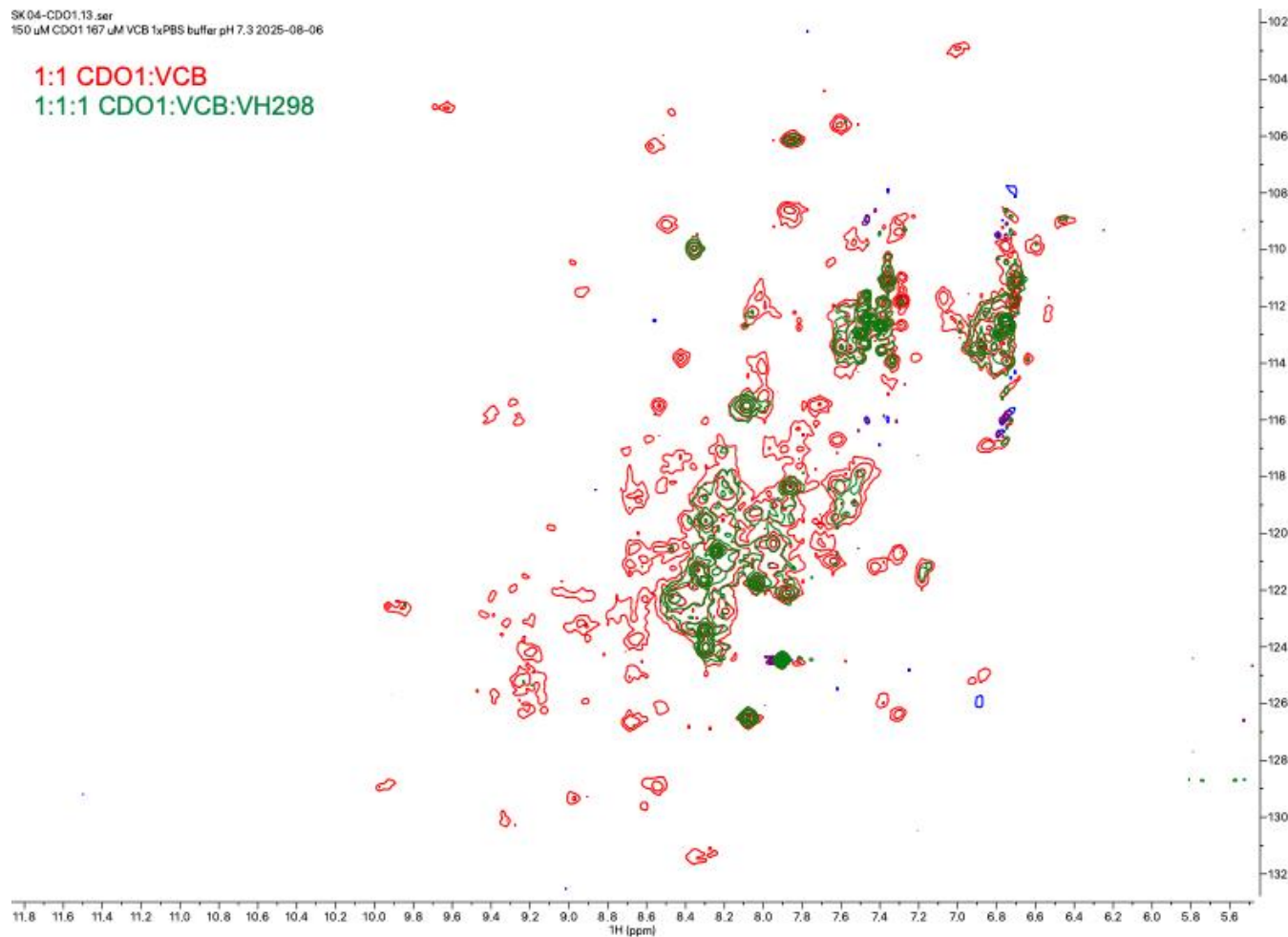


VH298

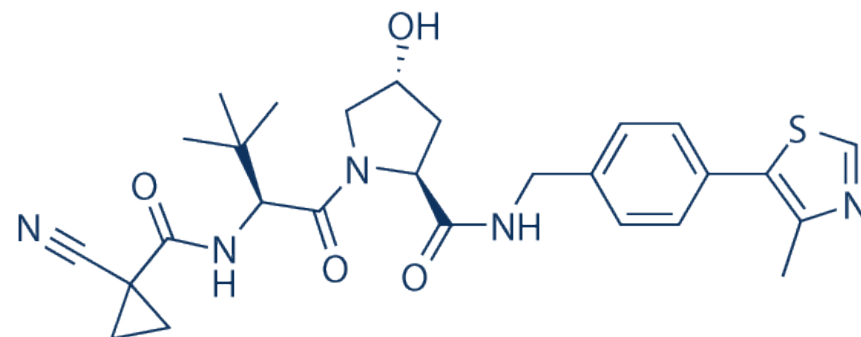
SK 04-CDO1.13.ser
150 μ M CDO1 167 μ M VCB 1xPBS buffer pH 7.3 2025-08-06

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

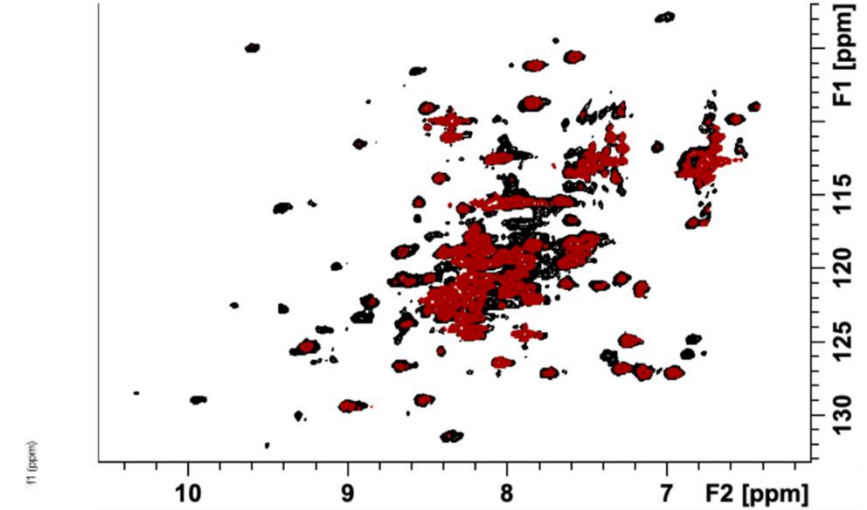
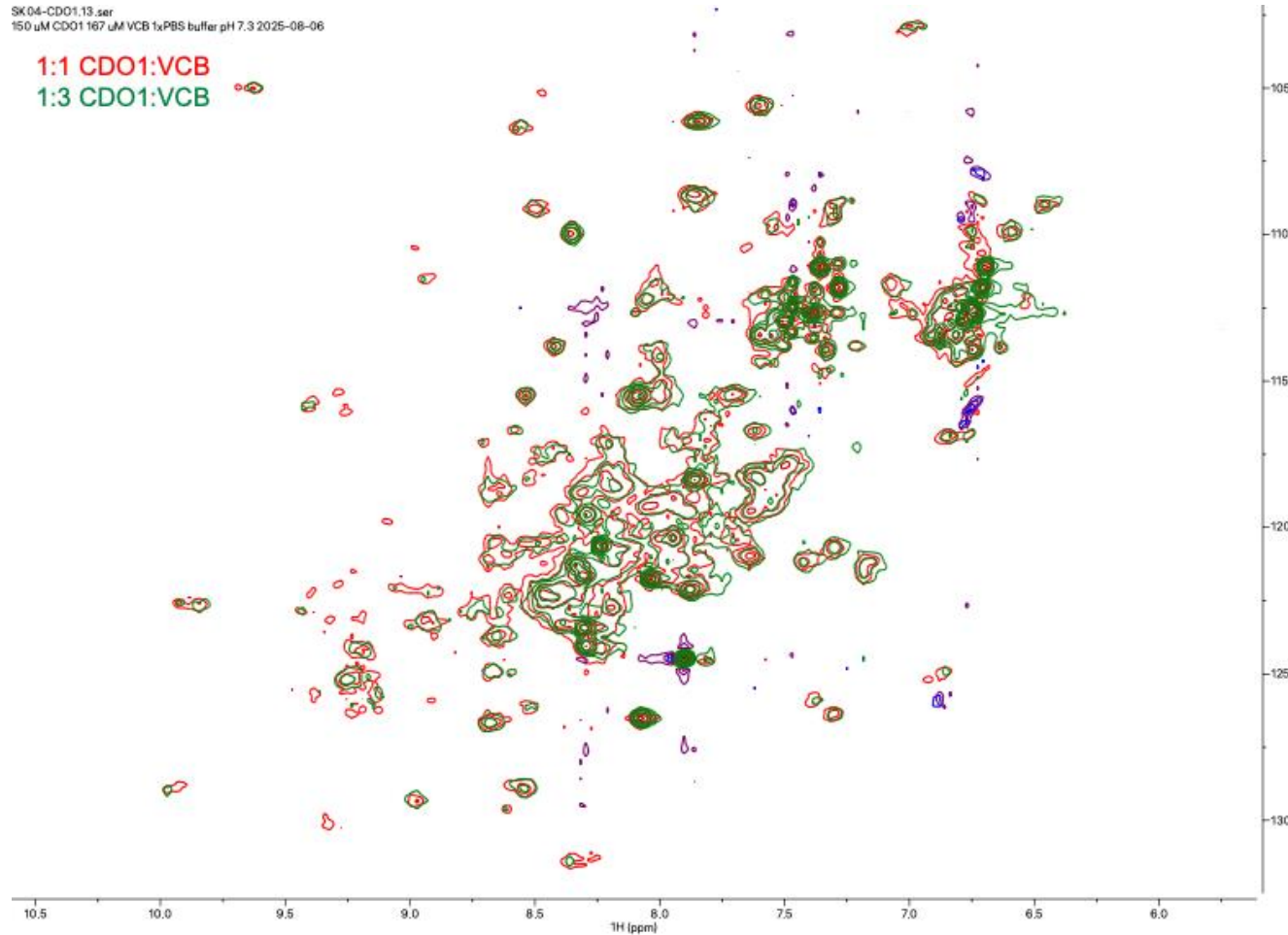


Figure 62: $^1\text{H}/^{15}\text{N}$ -HSQC of CDO1 with and without 2.8-fold excess of VCB.

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

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

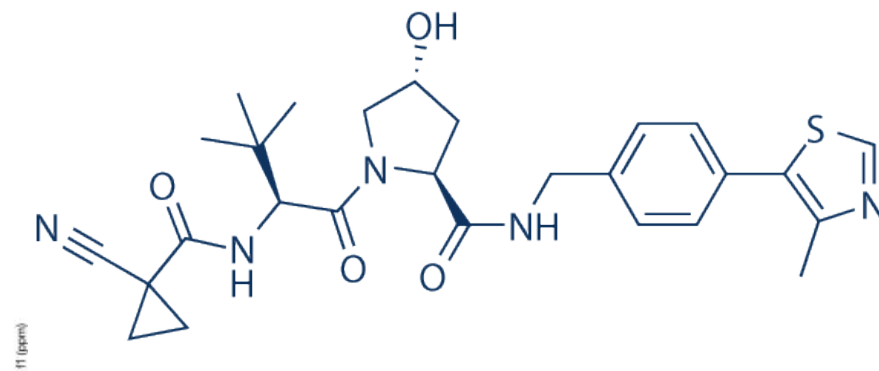


SK04-CD01_33_ser
150 μ M CD01 450 μ M VCB 1xPBS buffer pH 7.3 2025-08-08

1:3 CD01:VCB
1:3:3 CD01:VCB:VH298



1:3 CD01:VCB vs 1:3:3 CD01:VCB:VH298





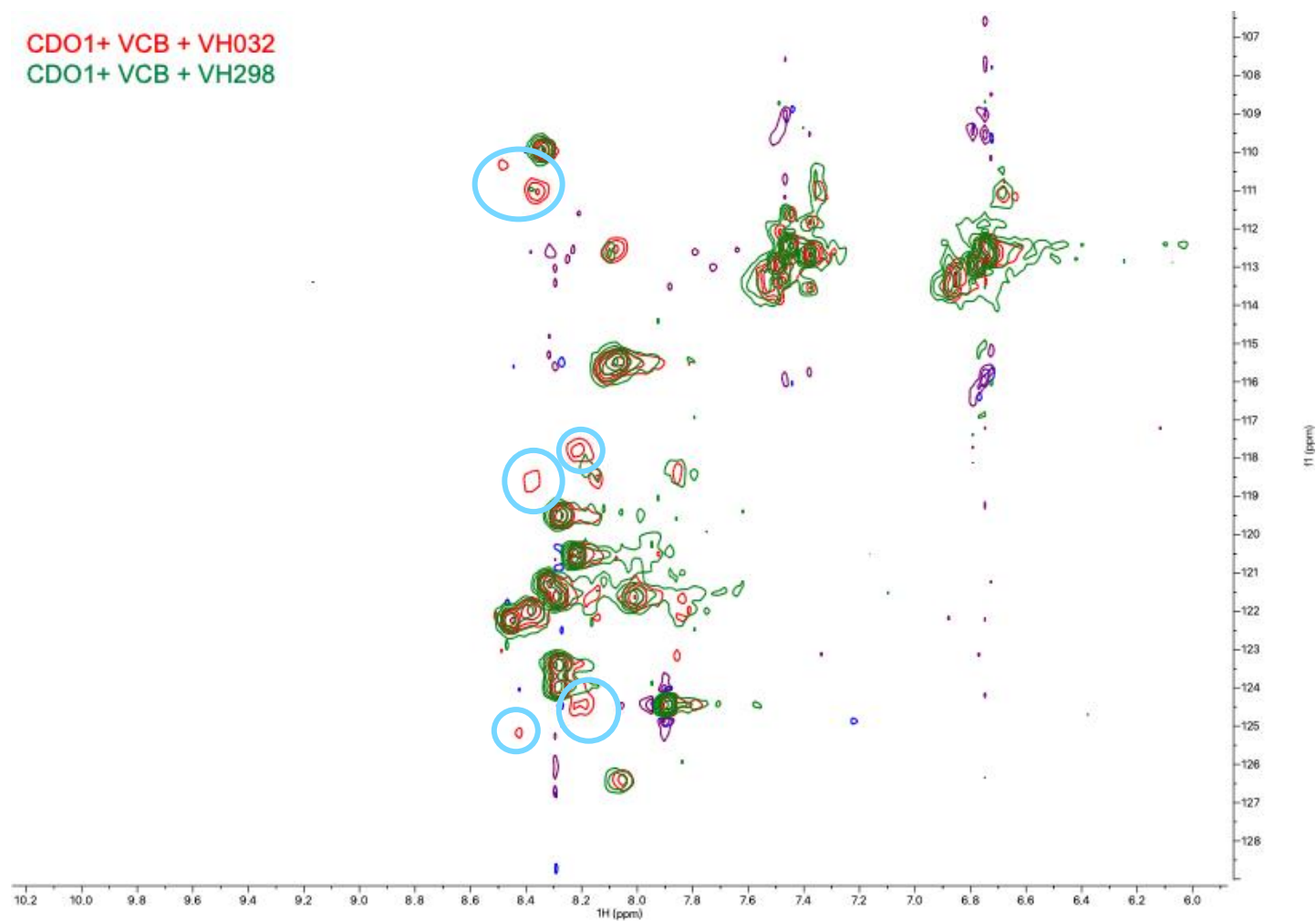
CDO1+ VCB + VH032
CDO1+ VCB + VH298



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

Personal Experiences



Centre for Targeted
Protein Degradation
University of Dundee

innovate
collaborate
inspire



Personal Experiences



Week 1



Personal Experiences



Week 1



Week 5



Personal Experiences

Bacterial cell culture

ÄKTA HPLC system

Manual column

NMR



Personal Experiences

Bacterial cell culture

Overnight culture

Using minimal media

Using big centrifuges

Monitoring OD

Making media

! CLEANING !

ÄKTA HPLC system

Cleaning the system

Loading samples

Dialysis

Running/scanning
Gels

Setting methods

Understanding the
machine

Nanodrop

Manual column

GST-tagged
recombinant protein

Cleaning/
re-equilibrating the
column

Data interpretation

Understanding the
machine

NMR

Making NMR samples

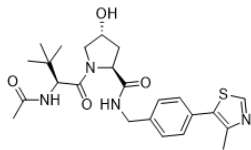
Data interpretation

Using the NMR
machine

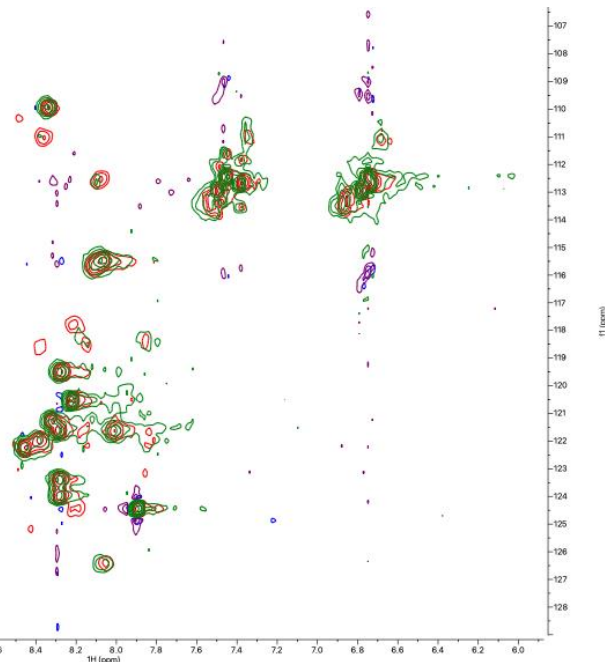
Lab notebook

Understanding
applications of NMR

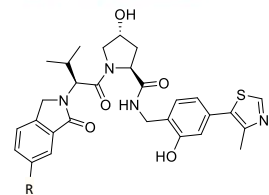
CDO1+ VCB + VH032
CDO1+ VCB + VH298



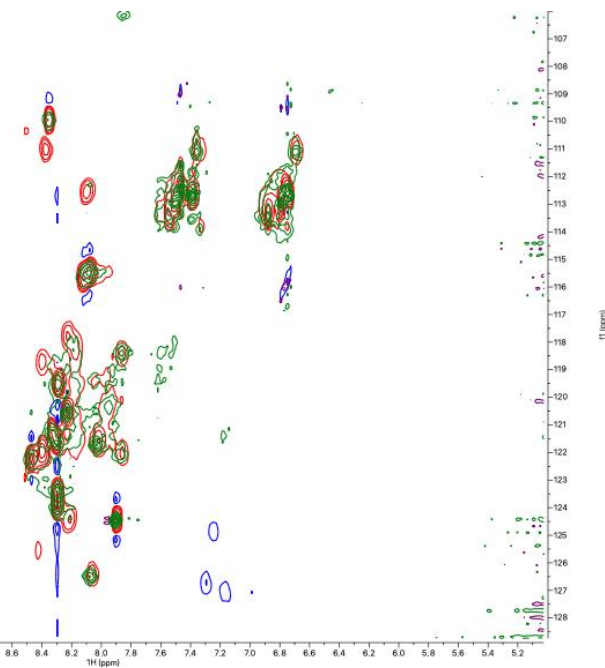
VH032



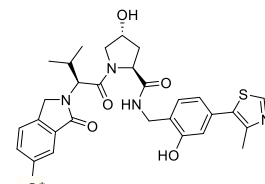
CDO1 + VCB + YC-XX1
CDO1+ VCB + VH298



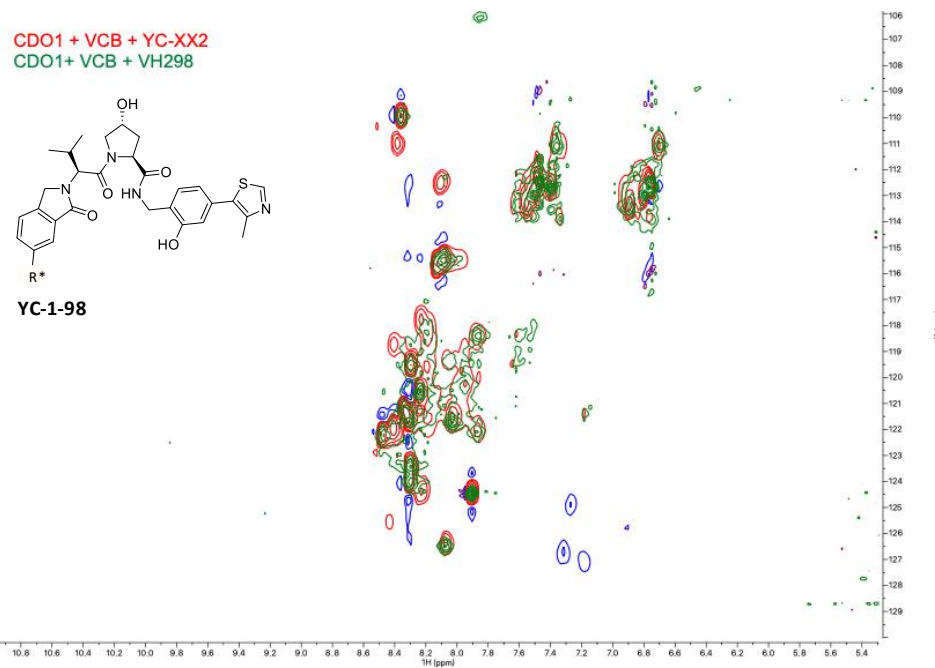
YC-22-1-15



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



YC-1-98



Centre for Targeted
Protein Degradation
University of Dundee

innovate
collaborate
inspire