Does VH298 glue CDO1 to VCB?

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Background

VCB is a trimeric complex consisting of Elongin B, Elongin C, and a substrate receptor; the von Hippel-Lindau protein (VHL). When bound to additional Cullin-2 and Rbx1 proteins, the full complex is an E3 ligase which ubiquitinates the substrate, leading to its proteasomal degradation of target proteins binding of VHL. The VHL ligand, **VH298** [1], was recently identified to glue CDO1 to VHL, leading to its degradation [2]. Structural insights of this interaction have yet to be revealed.

Project summary

Alongside known molecular glues for CDO1 such as **VH032**, Ciulli group has developed a series of their own hydroxyproline-based molecular glues. NMR had been used to confirm glueing for **VH032** and the lab's developed glues, showing similar glueing effects. **VH298**, however has not yet been characterised by NMR. My project will address this gap and allow the comparison of **VH298** to **VH032** and Ciulli group's glues. Remarkably, results revealed **VH298** inducing a different glueing interaction, setting it apart from the others studied by the lab.

Aims

To understand the effects of **VH298** on the interactions between VCB and CDO1, I planned to:

- Express VCB in *E. coli* and purify it in various chromatographic steps
- Express and purify CDO1 and ^{15}N-labelled CDO1 in *E. coli* using M9 medium
- Evaluate effect of **VH298** glue on interactions between VCB and CDO1 using 2D NMR.

Methods

Expression and purification of VCB: His-tagged VCB was expressed in *E. coli* in LB medium. Cells were induced with IPTG and grown overnight before harvesting by centrifugation, the pellets stored at -20ºC and benzonase. Thawed pellets were lysed using a cell disruptor, followed by syringe filtration. The filtered lysate was put through a nickel column and eluted with a 20-500mM imidazole gradient. The His-tag was cleaved with TEV protease, followed by reverse nickel column. Fractions containing VCB were pooled, dialysed, and run over an anion exchange column with a 0-500m NaCl gradient. The last gel filtration purification step yielded 20 aliquots of 20 µL (1.27mM) which were flash-frozen and stored at -80ºC.

Expression and purification of unlabelled and ^{15}N-labelled CDO1: GST-tagged CDO1 was expressed in E. coli in M9 minimal medium supplemented with either unlabelled or ^{15}N-labelled NH_4Cl as the nitrogen source, and ampicillin for selection. Expression was induced with IPTG followed by $Fe(II)SO_4$, and cells were harvested by centrifugation and frozen at –20 °C. Pellets were lysed with a cell disruptor then applied to glutathione–sepharose resin. After washing, GST tags were cleaved on-column with TEV protease, and the flow-through was concentrated and purified by gel filtration. This yielded 38 40µL aliquots at 370µM of ^{14}N-CDO1, and 2X 50µL aliquots of 1.38mM of ^{15}N-CDO1. The aliquots were flash-frozen and stored at -80ºC.

NMR: NMR samples were made by preparing a 1:10 dilution of the 1.38mM CDO1 stock in PBS buffer. VCB protein was buffer exchanged with PBS buffer. NMR analysis was performed on ^{15}N-CDO1. Produced spectra include 1:1 and 1:3 CDO1:VCB, 1:1:1 and 1:3:3 CDO1:VCB:**VH298**.

Results

Protein expression and purification

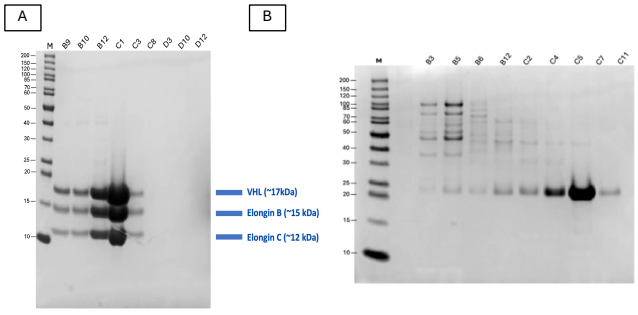

Figure 1. SDS-PAGE of Size Exclusion Chromatography fractions obtained from (A) VCB (44kDa) purification and (B) ^{15}N-CDO1 (23kDa) purification.

Overall, expression and purifications yielded 87mg of VCB, 13mg of unlabelled CDO1, and 3mg of ^{15}N-CDO1.

2D NMR

To assess potential protein–protein interactions, ^{1}H/^{15}N HSQC NMR spectra of CDO1 were acquired under different ratios and compared. Loss of NMR signal intensity was monitored, as such loss indicates the corresponding residue is involved in ternary complex formation.

Spectra superimposition of free CDO1 and the 1:1 mixture of CDO1+VCB (Figure 2) revealed no significant changes, indicating CDO1 does not intrinsically interact with VCB. Upon addition of **VH298** (1:1:1 ratio), signal loss was observed (Figure 3), demonstrating that ligand binding is required for complex formagtion. This effect was enhanced with a 1:3:3 ratio of VCB:CDO1:**VH298** (Figure 4).

Previously studied glues including **VH032** all display the same interaction by NMR. Here, I compare my **VH298** spectra to **VH032** as it is representitive of all other glues. Figure 5 shows that both compounds engage with CDO1, however some signals are loss only with **VH298**. This suggests that **VH298** induce slightly different interactions which **VH032** does not.

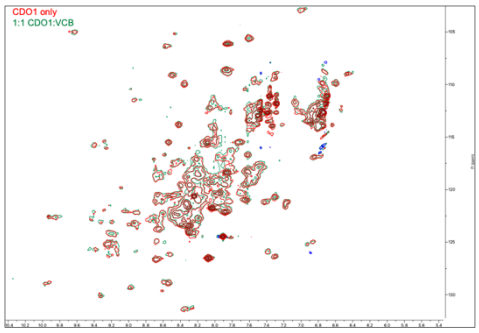

Figure 2. $^{1}H/^{15}N$-HSQC of CDO1 with vs. without 1:1 VCB

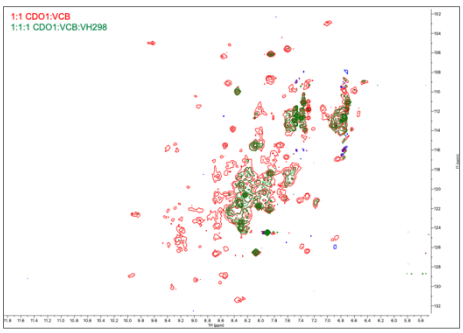

*Figure 3. $^{1}H/^{15}N$-HSQC of 1:1 CDO1 + VCB vs. 1:1:1 with **VH298**.*

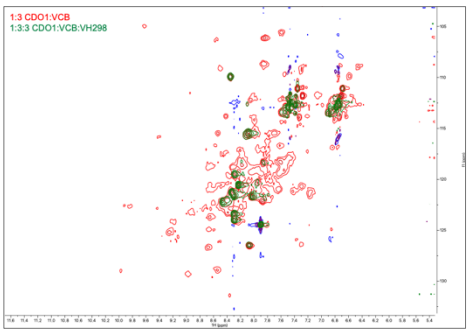

*Figure 4. $^{1}H/^{15}N$ -HSQC of 1:3 CDO1 + VCB vs. 1:3:3 with **VH298**.*

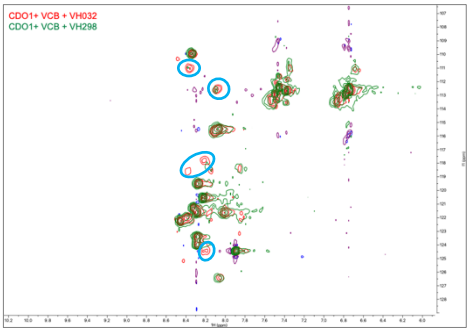

*Figure 5. $^{1}H/^{15}N$-HSQC of 1:3:3 CDO1:VCB: **VH298** vs 1:1:1 CDO1:VCB: **VH032***

Future Directions

- *Backbone resonance assignment*: Assign each signal in the NMR spectra to a specific residue in CDO1. This will identify which residues are directly involved in the glueing interaction.
- *Control NMR experiment*: Analyse CDO1 with **VH298**, but without VCB to confirm that observed signal loss is solely due to CDO1-**VH298**-VCB interactions.
- *Structure characterisation*: Solve the 3D structure of the complex to pinpoint key interactions for complex formation. Use methods such as X-ray crystallography, Cyro-EM, NOESY-based NMR.
- *Binding affinity*: Analyse the affinity of complex formation for **VH298** and other glues.

Departures from original plan

My original plan involved using ITC to investigate binding affinity **VH298** to VCB and CDO1. The machine became unavailable, so I did not have the chance to carry this out. With my extra time, I purified extra VCB for the lab, and optimised CDO1 expression conditions.

Value of the studentship

To the student

Working independently in the lab improved my confidence, allowed me to overcome weaknesses in my practical work, and equipped me with technical skills including protein expression and purification, and NMR sample preparation and analysis. I developed transferrable skills in time management, independent learning, and record-keeping.

To the group

My work completed the group's investigation of a series of CDO1:VHL molecular glues. My finding that **VH298** appears to induce a slightly different interaction site than the other glues is highly valuable. Furthermore, I produced purified VCB and CDO1 that were subsequently used for SPR experiments, further contributing to the group's ongoing research.

To the Biochemical Society

This studentship demonstrates the society's success in inspiring scientific careers and supporting early-stage researchers. By providing a valuable insight into academic research, the Biochemical Society has directly contributed to my development as a future scientist.

Figure 6. Student Sansara Klinsukont with supervisors, Dr. Lianne Wieske and Prof. Alessio Ciulli.

Acknowledgments

I am grateful to the Biochemical Society for their support. This experience has deepened my passion for research and sparked strong motivation to pursue a scientific career. I was able to acquire vital laboratory and research skills which I would not have encountered during my degree, including protein expression and purification and NMR spectroscopy. I would also like to express my sincere appreciation to Prof. Alessio Ciulli for the opportunity and guidance, Dr Lianne Wieske who supervised me daily, and everyone at the lab for providing invaluable support throughout the project.

References

[1] J. Frost *et al.*, "Potent and selective chemical probe of hypoxic signalling downstream of HIF-α hydroxylation via VHL inhibition," *Nature Communications 2016 7:1*, vol. 7, no. 1, pp. 1–12, Nov. 2016, doi: 10.1038/ncomms13312.

[2] A. Tutter *et al.*, "A small-molecule VHL molecular glue degrader for cysteine dioxygenase 1," *Nat Chem Biol*, pp. 1–9, Jun. 2025, doi: 10.1038/S41589-025-01936-X;SUBJMETA=1266,507,535,609,613,631,92;KWRD=MECHANISM+OF+ACTION, SCREENING, SMALL+MOLECULES, X-RAY+CRYSTALLOGRAPHY.