Reviewing the Crystal Structures of Ternary Complexes Formed by Heterobifunctional Degraders

Heterobifunctional degraders rely on the formation of productive ternary complexes to activate cellular degradative machinery, causing the proteolysis of a protein of interest (POI).[1] Typically, the POI is tethered to an E3 ubiquitin ligase protein, which, downstream, leads to the degradation of the POI via the ubiquitin degradation pathway (Figure 1). These ternary complexes are dynamic in nature, and various differing protein–protein conformations can lead to a degradation event. This poses a unique challenge for structure-based drug design, as there are multiple correct solutions for the protein–protein conformations.

Whilst crystallographic structures of these complexes were once rare, their availability has steadily increased over the years. Scientists have collated a database of publicly available heterobifunctional degrader-containing ternary complex crystal structures.[2] Through careful analysis of these structures, the biological assemblies of these complexes were investigated, with the aim of discovering whether these assemblies provide information on the viability of ternary complex conformations induced by heterobifunctional degrader molecules.

Protein crystallography produces solid-state assemblies of molecules, which can come in different forms. One of the challenges of computational drug design is deducing whether these assemblies are biologically relevant. In particular, crystal packing forces can distort or otherwise alter the configuration of the ternary complex. Examination of the crystallographic symmetry mates of heterobifunctional molecule-containing ternary complexes can help determine whether this is the case. All modelling and analysis were performed using Flare™.[3]

For this investigation, the analysis initially focused on the well-known and studied VCB–BRD4(BD2) ternary complexes. VCB consists of the Von Hippel–Lindau E3 ligase (pVHL), along with Elongin B and Elongin C, which are key components of the larger Cullin 2-RING ligase complex (CRL2). The formation of the CRL2 complex and eventual tagging of accessible lysines on the POI is a key mechanism of degradation in this process.[4]

Through searching the literature and the Protein Data Bank (PDB), nine VCB–BRD4(BD2) complexes were identified. Of these, three were extended CRL2 complexes. The six ternary complexes were first compared (Figure 2).

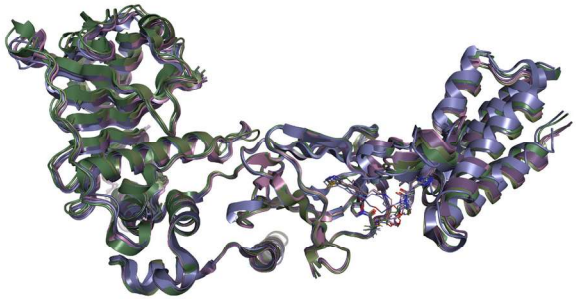

Figure 2. PDB codes: 8BDT, 5T35, 8BDX, 7ZNT, 6SIS and 8YMB. These structures were aligned and superposed to pVHL. The BRD4(BD2) protein is depicted in the alpha helices on the right of these structures.

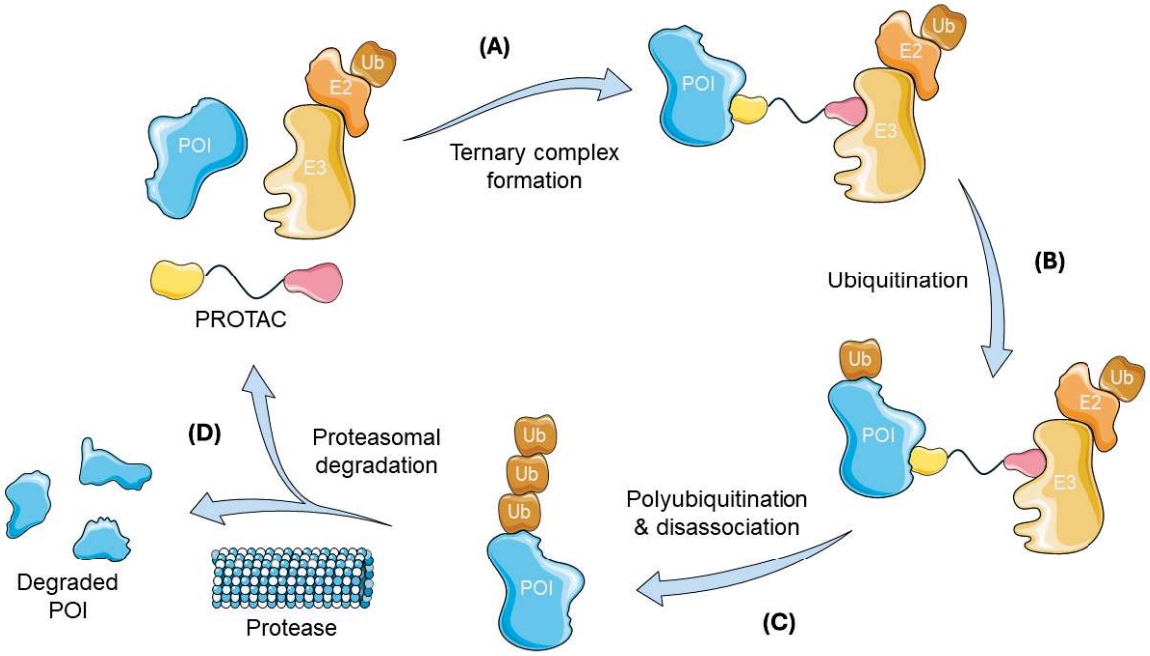

Figure 1. The ubiquitination cycle, showing the recycling of a heterobifunctional degrader and proteolysis of the POI. (A) is the POI binder of the degrader binding to the POI, whilst the E3 binder of the degrader binds to the E3 ligase. (B) The POI–degrader–E3–E2–Ub ternary complex forms. (C) The POI is polyubiquitinated by the intermediate complex, marking it for degradation by the proteosome. (D) The POI is degraded and the heterobifunctional degrader molecule is released, allowing it to act again. This figure was partly generated using Servier Medical Art, provided by Servier, licensed under a CC BY 4.0 license.

By aligning the sequences of pVHL and superposing these complexes, it can be observed that the 3D conformation of the ternary complexes is well conserved. This suggests a strongly preferred protein–protein binding mode between pVHL and BRD4(BD2), regardless of the heterobifunctional degrader structure.

A key focus of this work was the investigation of crystallographic symmetry structures. By generating the crystal mates from the PDB structures, it was found that five of the six ternary structures formed dimers, whilst only one had a monomeric structure. Observing the interacting faces of the dimeric structures revealed a strong interaction between the dimeric face of pVHL opposite to the face of ligand binding (L129–L153). This interaction appears strongly conserved between the five ternary structures showing this dimerisation. Numerous hydrogen bonds and hydrophobic interactions are present when these pVHL faces are oriented to depict dimeric assembly.

In contrast, when the monomeric assembly is examined, this interaction does not occur. This raises the question of why this difference exists and whether it has any biological consequences.

To investigate this, the available extended CRL2 complexes were superimposed with the proteins of a dimeric crystal structure and the monomeric crystal structure, aligning and superposing to the pVHL protein. This provided several observations. First, the open, non-crosslinked extended CRL2 complex showed minimal clashes between the extended complexes. Where minor clashes did occur, these were within the BRD4(BD2) component of the structures, a dynamic part of the system. Notably, it is conceivable that both extended structures feature sufficient flexibility and available space between the BRD4(BD2) and RBX proteins to allow recruitment of the E2 ligase and conjugation of ubiquitin, leading to a degradation event (Figure 3).

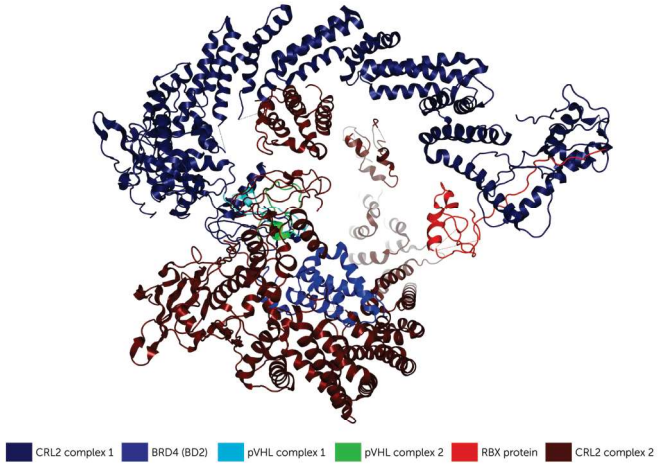

Figure 3. Depicting the formation of a supercomplex from dimer complexes, suggesting they may be biologically relevant. Extended CRL2 complex (8RWZ), duplicated, aligned and superposed to the opposite dimeric pVHL protein (7ZNT), where CRL2 complex 1 is dark blue and CRL2 complex 2 is dark red. The dimeric faces of pVHL are shown in cyan (complex 1) and green (complex 2). The BRD4(BD2) protein (blue), the RBX protein (red).

In contrast, when the two cross-linked extended CRL2 complexes were examined, major clashes were observed. These clashes occurred in conformationally dynamic features of the system, namely the E2 ligase and ubiquitin proteins.

The same process was repeated with the monomeric ternary complex. In this case, severe clashes were observed among many of the conformationally inflexible proteins of the extended structure (Figure 4).

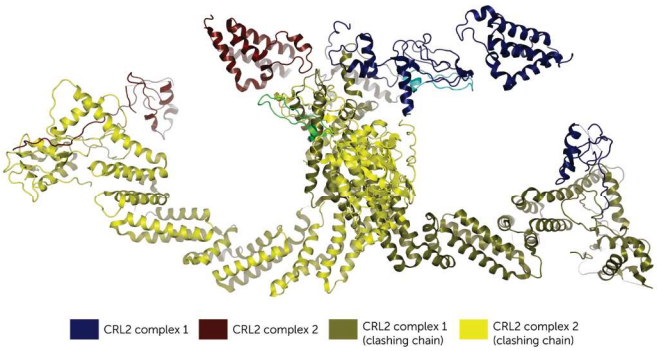

Figure 4. Demonstrating a different crystal packing. Extended CRL2 complex (8RWZ), duplicated, aligned and superposed to neighbouring monomeric pVHL proteins (8YMB), where CRL2 complex 1 is dark blue and CRL2 complex 2 is dark red. Clashes are observed between protein complexes 1 (dark yellow) and 2 (light yellow). This indicates that the supercomplex cannot form and is therefore biologically irrelevant.

This presents several challenges. Dimeric extended complexes appear to be conformationally possible. However, these extended complex dimers are not depicted in the electron density maps provided with the structures. A key caveat is that these extended complexes were generated using cryo-EM methods, which differ from the X-ray crystallography methods used for the ternary structures. Crystallographic conditions and methodologies can provide different views of these dynamic structures, and it would be unwise to assume that the extended dimer is impossible based on the limited information currently available, particularly without confirmation via an orthogonal method.

Additionally, the monomeric ternary complex still leads to a degradation event. This indicates that this crystallographic assembly is as relevant as the others, despite being the sole example available. This raises the possibility that both dimeric and monomeric assemblies can lead to degradation events and that one may not necessarily be more productive than the other. This observation warranted further investigation.

The available crystal structures of VCB–SMARCA2 hetero-bifunctional degrader complexes were examined next. As before, alignment was performed on the E3 ligase; however, in this instance, Flare's Match-3D superposition tool was used to improve alignment in three-dimensional space. These systems exhibited a wide range of binding conformations (Figure 5).

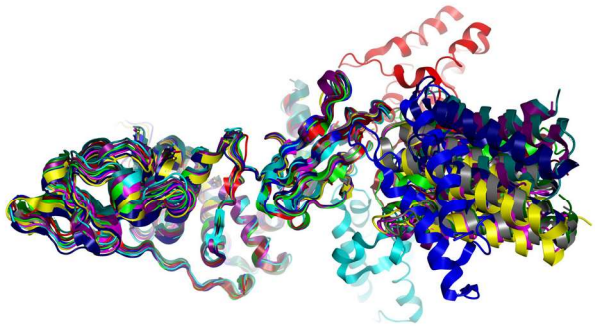

Figure 5. VCB–SMARCA2 complexes, PDB codes 7Z76 (dark red), 7Z77 (dark blue), 7Z6L (purple), 6HAY (yellow), 7S4E (green), 6HAX (grey), 9DTY (red), 9MR9 (blue), 9HYB (bright green), 9HYN (pink), 9HYO (light blue) and 9HYP (teal) aligned on pVHL and superposed with Flare's Match-3D.

Figure 5 shows that whilst the VCB complex (pVHL–Elongin C–Elongin B) is well conserved across all examined PDB structures (the beta-pleated sheets on the left-hand side), the SMARCA2 components adopt a broad range of conformations (the alpha-helices on the right-hand side). Examination of the crystal symmetry mates of a subset of these VCB–SMARCA2 complexes (9HYB, 9HYN, 9HYO and 9HYP), deposited as part of the same publication,[5] revealed further complexity. Structures 9HYO and 9HYP exhibited monomeric crystallographic units. In contrast, 9HYB and 9HYN formed dimeric units, with significant interactions occurring between pVHL molecules. This behaviour appeared unrelated to the ternary poses displayed. This observation is particularly notable given that the same crystallographic conditions were reported for the generation of all X-ray crystal structures in this series.

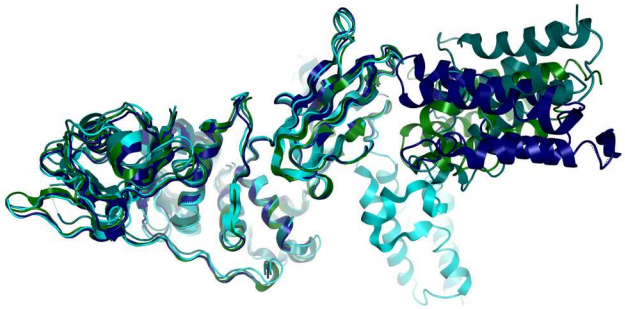

Figure 6. VCB–SMARCA2 complexes, PDB codes 9HYB (green), 9HYN (teal), 9HYO (cyan) and 9HYP (dark blue) aligned on pVHL and superposed with Flare's Match-3D.

Figure 6 shows structures 9HYO (cyan) and 9HYN (teal). The pose of 9HYN closely aligns with that of 9HYB (green). Despite this similarity in three-dimensional conformation, the crystal symmetries differ. Confidence in these differing crystal symmetries is supported by examination of the electron density around pVHL in the 2Fo–Fc electron density maps within Flare. Consideration of the electron density and inspection of the pVHL dimeric interaction interface indicate that although the 3D structure differs in this region, these differences do not explain whether crystal mates adopt monomeric or dimeric assemblies. Furthermore, no clear correlation was observed between heterobifunctional degrader poses and monomeric or dimeric crystal structures.

The conclusion of this study was that the biological assemblies observed in deposited crystal structures appear to be unrelated to degradation efficiency and cannot be reliably determined based solely on the conformation of ternary complexes.

However, a potential conformation of dimeric poses for extended CRL complexes was identified that could plausibly lead to degradation events. As additional extended CRL complexes become available, further investigation may extend these findings. This remains an area of active interest in heterobifunctional degrader research. The increasing availability of structural data provides unprecedented opportunities to study these dynamic processes and may ultimately help resolve mechanisms that are currently poorly understood.

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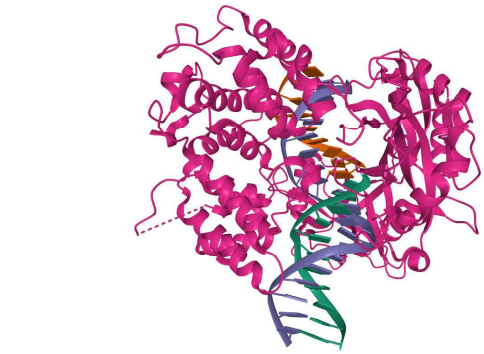

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