Rational Design of Molecular Glues: Breakthroughs and Perspectives

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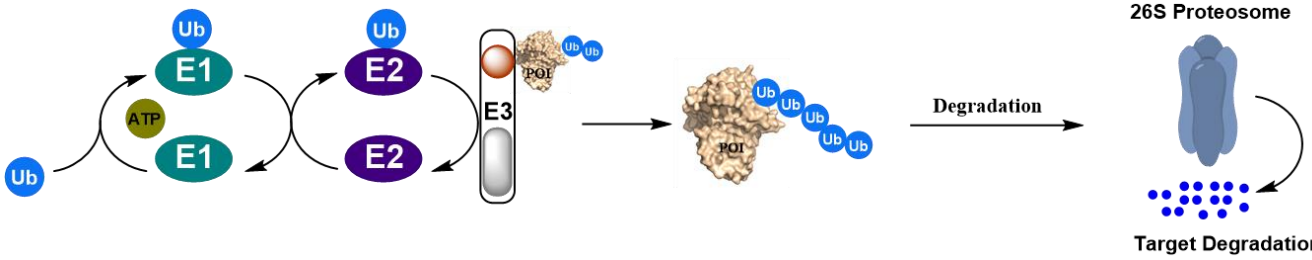
Abstract

CRBN is a substrate receptor for the Cullin Ring E3 ubiquitin ligase 4 (CRL4) complex. It has been observed that CRBN can be exploited by small molecules to facilitate the recruitment and ubiquitination of non-natural CRL4 substrates, resulting in the degradation of neo-substrate through the ubiquitin-proteasome system. This phenomenon, known as molecular glue-induced protein degradation, has emerged as an innovative therapeutic approach in contrast to traditional small-molecule drugs. One key advantage of molecular glues, in comparison to conventional small-molecule drugs adhering to Lipinski's Rule of Five, is their ability to operate without the necessity for specific binding pockets on target proteins. This unique characteristic empowers molecular glues to interact with conventionally intractable protein targets, such as transcription factors and scaffold proteins. The ability to induce the degradation of these previously elusive targets by hijacking the ubiquitin-proteasome system presents a promising avenue for the treatment of recalcitrant diseases. Nevertheless, the rational design of molecular glues remains a formidable challenge due to the limited understanding of their mechanisms and actions. This review offers an overview of recent advances and breakthroughs in the field of CRBN-based molecular glues, while also exploring the prospects for a systematic approach to designing these compounds.

Background

The ubiquitin-proteasome system (UPS) plays an important role in varieties of cellular processes including cell-cycle regulation, endocytosis, DNA repair, gene transcription and autophagy. [1,2] UPS system usually depletes proteins that are damaged or folded mistakenly in cells to maintain the regular cellular activity. To degrade the target protein, UPS system mainly goes through three processes (Scheme 1) [2]: 1) Ubiquitin activation by E1 enzymes assisted with adenosine triphosphate (ATP); 2) The activated ubiquitin is transferred to the target protein with covalent bond by conjugating (E2) and ligating (E3) enzymes; 3) The target protein tagged with ubiquitin is recognized and degraded by 26S proteasome.



Scheme 1. Target protein degradation process through UPS system (Ub: Ubiquitin; E1: Ubiquitin-activating enzyme; E2: Ubiquitin-conjugating enzymes; E3: Ubiquitin-ligase enzymes; POI: Protein of Interest; 26S proteasome: A 2.5-MDa proteolytic protein complex that controls protein homeostasis and specific cellular process in all eukaryotes).

UPS has emerged as a groundbreaking platform for a novel therapeutic approach, known as UPS-based precision therapeutics (UPT). This approach represents a revolutionary shift from traditional small-molecule drugs, as it focuses on the precise elimination of specific cellular proteins of interest (POI) rather than merely modulating their partial functions [3].There are two major strategies within the realm of UPT that have gained prominence and become potent tools in the treatment of various diseases through target protein degradation (TPD):1)PROTAC (Proteolysis-Targeting Chimeras): PROTACs are molecular constructs designed to recruit target proteins for degradation. They work by linking the target protein to an E3 ubiquitin ligase, marking it for ubiquitination and subsequent degradation by the proteasome [4]. This approach has the potential to target a wide range of disease-associated proteins. 2)Molecular Glue Degrader: Molecular glue degraders

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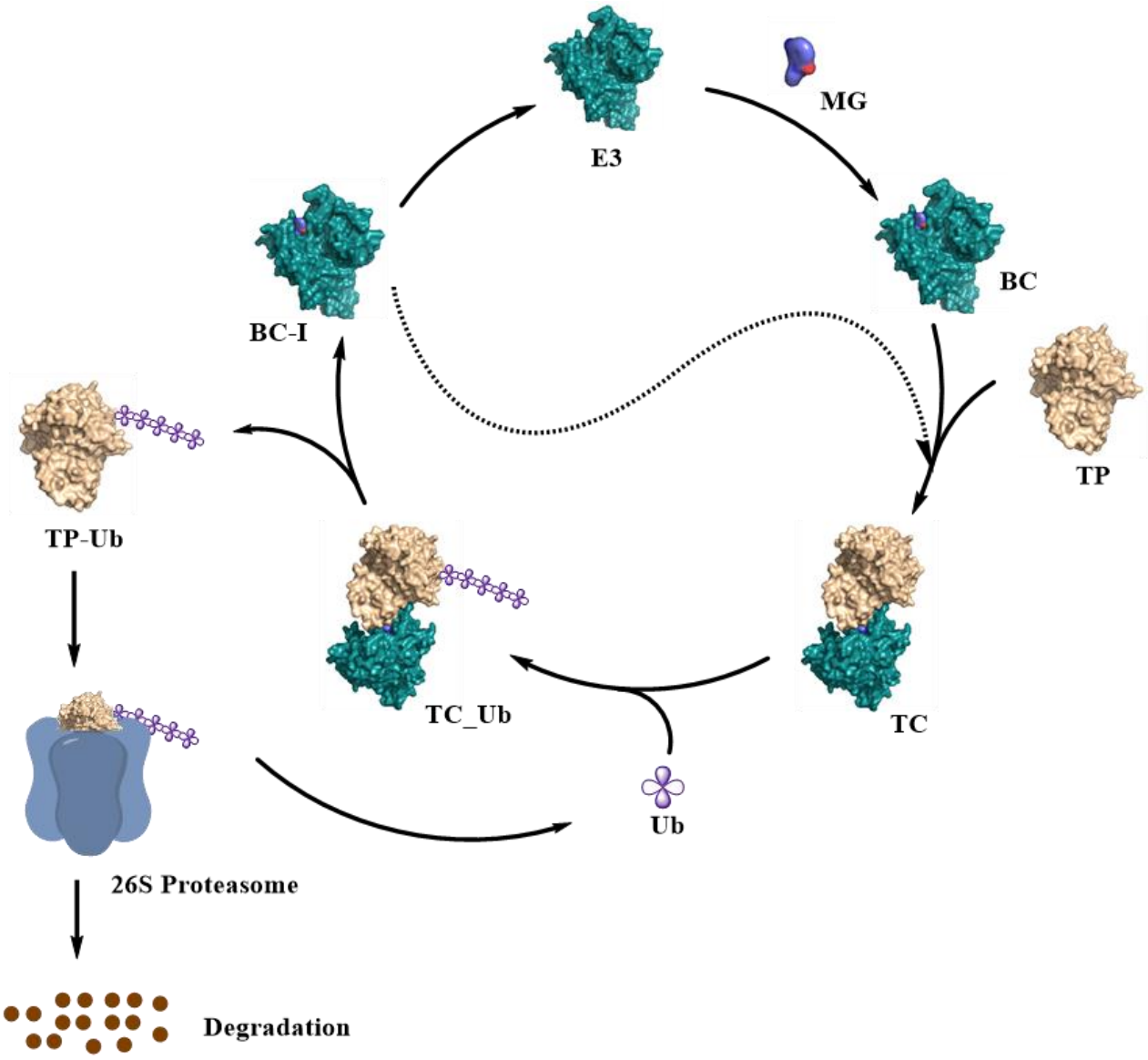
are small molecules that exploit the cellular machinery to induce the ubiquitination and degradation of specific target proteins. As mentioned earlier, this approach is valuable in targeting proteins previously deemed "undruggable" because it does not rely on specific binding pockets on the target proteins [5]. These TPD modalities, like PROTACs and molecular glue degraders, open the door to an expanded range of potential therapeutic targets, as they can be applied to more than 80% of proteins that were traditionally considered undruggable. This shift in strategy has the potential to usher in a new era of precision medicine by allowing for highly specific and effective treatments for a wide array of diseases.

The concept of PROTAC technology was originally proposed in 2001[6]. PROTACs are heterobifunctional small molecules composed of three essential components: two ligands, one that binds to the target protein of interest (POI) and the other to an E3 ligase, and a linker that connects these two ligands. Over the past two decades, PROTAC technology has transitioned from academic research to industry application. Several prominent biotechnology companies, including Arvinas, Bristol Myers Squibb (BMS), Nurix Therapeutics, and Kymera Therapeutics, have heavily invested in the development of PROTACs. Arvinas, for instance, successfully advanced the first PROTAC molecules, ARV 110 and ARV471, to phase II clinical trials in 2020 and 2021, respectively. Despite promising results in clinical studies, PROTACs continue to face several significant challenges, one of which is their relatively high molecular weight leading to low cell permeability. Addressing this issue is crucial for enhancing the effectiveness of PROTACs as therapeutic agents and further expanding their potential in precision medicine.



Scheme 2. Dynamic Cycle of Molecular Glue induced target protein degradation (E3: E3 ligase; MG: Molecular glue; BC: Binary complex; TP: Target protein; TC: Ternary complex; Ub: Ubiquitin; TC_Ub: Ubiquitinated ternary complex; TP_Ub: Ubiquitinated target).

Molecular glues (MGs) offer a complementary approach to address the issues of low druggability encountered with PROTACs. Molecular glues are monovalent molecules that facilitate the recruitment of target proteins for degradation by forming a novel interaction with E3 ligase[7], as illustrated in Scheme 2. The process involves the following steps: 1) MG Interaction with

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E3 Substrate Receptor ($E3^{SR}$): Initially, a specific MG interacts with the substrate receptor of an E3 ligase, leading to the formation of a binary complex (BC). 2) Recruitment of POI: The newly formed surface in the binary complex recruits the protein of interest (POI), resulting in the formation of a ternary complex (TC). 3) Ubiquitination and Degradation: Once the ternary complex is established, the target protein is ubiquitinated directly by the E3 ligase. The ubiquitinated target protein is subsequently recognized and degraded by the 26S proteasome system. This approach ultimately leads to the elimination of the disease-causing protein target, offering a potential therapeutic strategy for addressing the underlying causes of specific diseases. By employing molecular glues, it becomes feasible to target a broader range of disease-associated proteins, including those previously considered undruggable, and to offer innovative treatment options.

Molecular glues indeed offer numerous advantages, including ease of preparation compared to PROTACs, lower molecular weight, and higher druggability. However, their discovery has presented significant challenges, especially when it comes to the rational design of molecular glues for specific targets. Thus far, most molecular glues have been identified serendipitously. Nevertheless, scientists are actively working on transitioning from serendipitous discovery to a more systematic and rational design approach [5h, 8,32]. In the context of this review, the development and the potential rational design tactics of molecular glues will be discussed. The structure of the review will be organized around the molecular glue structure and the crystal structure of the ternary complex, providing insight into the structural basis of their function and design.

Additionally, the review will explore potential design strategies for molecular glues based on CRBN, offering a detailed look into how these molecules can be systematically engineered to target specific proteins. This represents a significant step forward in harnessing the full potential of molecular glues as a therapeutic modality and overcoming the current challenges associated with their discovery and design toward specific disease-causing proteins.

$CRL4^{CRBN}$-Based Molecular Glue Development

Cereblon (CRBN) [9] serves as a crucial substrate receptor within the Cullin Ring E3 ubiquitin ligase 4 (CRL4) complex, comprised of DDB1, CUL4, and RBX1. This E3 ligase complex is particularly significant in the field of molecular glue development and is widely utilized in this context among more than 600 known E3 ligases [10]. The pioneering molecular glue that harnessed CRBN for the degradation of target proteins is thalidomide [11]. Thalidomide has been effective in the treatment of various medical conditions, including erythema nodosum leprosum, a complication of leprosy, and multiple myeloma [11,12]. However, it is essential to note that thalidomide is teratogenic for its association with birth defects when used by pregnant women. In an effort to develop more effective degraders with fewer side effects, Müller and his colleagues [13] embarked on a quest to discover more potent degraders, using thalidomide as a starting point. This was initiated even though the mechanism of thalidomide's action remained elusive at that time. Encouragingly, their research led to the identification of CC-5013 (Lenalidomide) and CC-4047 (Pomalidomide) as MGs with increased efficacy [14].

Lenalidomide (Figure 1c) was derived by introducing an amino group at the C6 of the phthaloyl section while removing a carbonyl group from the phthaloyl ring of thalidomide. On the other hand, Pomalidomide (Figure 1d) was developed by introducing only one amino group at the C6 position of the phthaloyl ring of thalidomide. Thalidomide, Lenalidomide, and Pomalidomide are collectively referred to as immunomodulatory imide drugs (IMiDs) [14]. These compounds represent a significant advancement in the field of molecular glue-based therapies, offering improved efficacy and selectivity while minimizing adverse effects.

The Handa group's groundbreaking work in 2010 marked the first discovery that CRBN was the direct target of thalidomide [15]. Subsequently, in 2014, the Thomä group obtained crystal structures of the DDB1-CRBN-E3 ligase complex in complex with thalidomide, lenalidomide, and pomalidomide. This crystallography work provided direct confirmation that CRBN is indeed the target of immunomodulatory imide drugs (IMiDs).

The crystal structures of CRBN interacting with IMiDs clearly revealed a common binding pattern. IMiDs occupy a shallow pocket on the CRBN surface, which is formed by residues N353, P354, H359, H380, W382, W388, W402, and F404 (Figure 1a, 1f). The nitrogen on the glutarimide ring of IMiDs forms a hydrogen bond with His380, acting as a hydrogen bond donor (Figure 1f). Additionally, the adjacent carbonyl group (Figure 1f) forms a second hydrogen bond as a hydrogen bond acceptor with Trp382. The lactam group on the glutarimide ring delocalizes to be coplanar with W382, while the remaining part of the glutarimide ring (C3-C4-C5) engages in van der Waals contacts and hydrophobic interactions with W382, W388, W402, and F404 (Figure 1f). Furthermore, one of the carbonyl groups from the phthaloyl ring interacts with Asn353, acting as a hydrogen bond acceptor. These structural insights have successfully elucidated the molecular-level mechanism of how IMiDs

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interact with the CRL4-CRBN E3 ligase. Several subsequent studies have further verified that IMiD drugs function by hijacking the CRL4-CRBN E3 ligase to degrade transcription factors like IKZF1 and IKZF3 [16]. In 2015, the Benjamin L. Ebert group

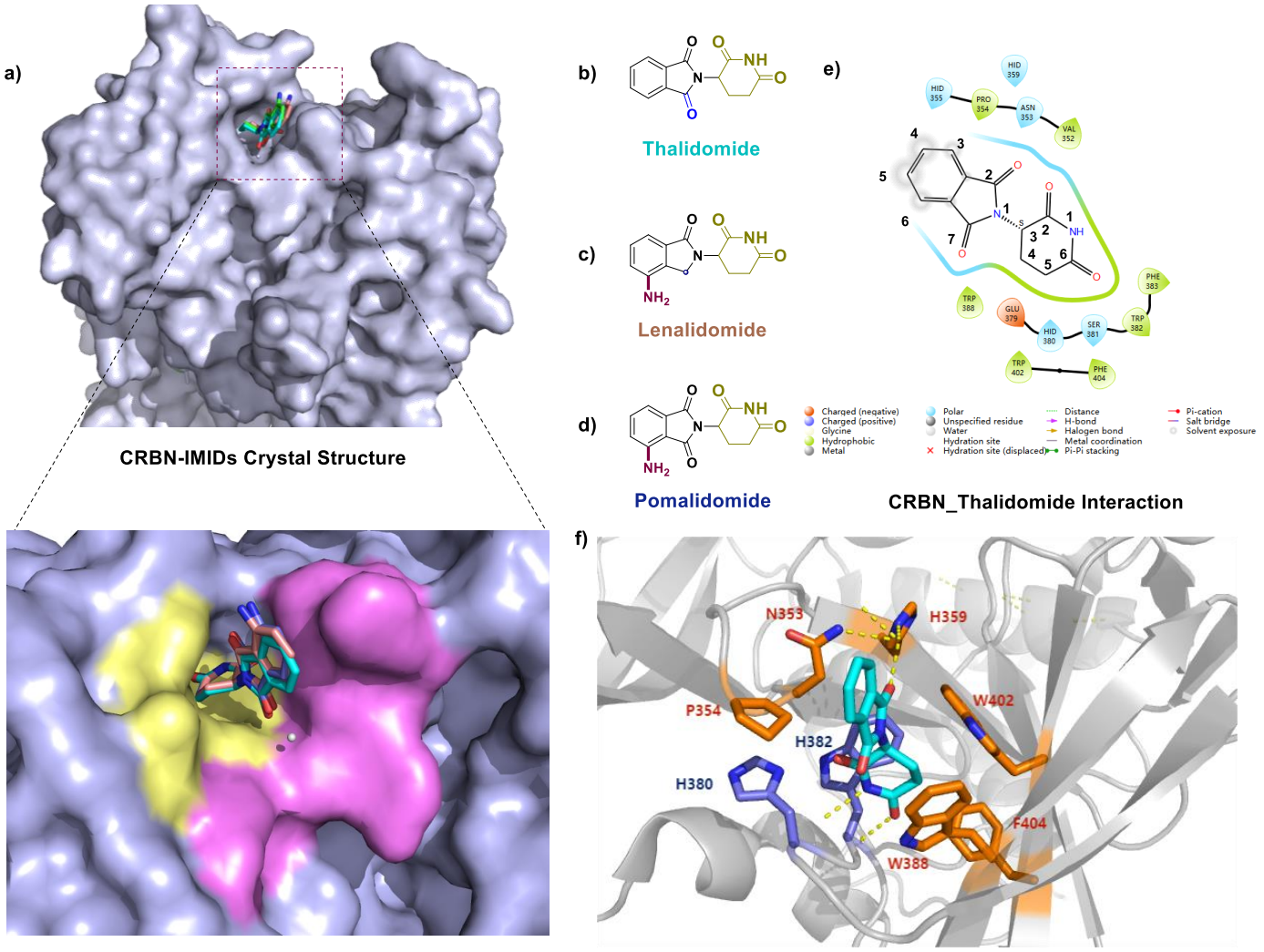

Figure 1. The crystal structure of CRBN with IMIDs compounds. a). The crystal structure of CRBN-IMIDs (PDB: 4CI1,4CI2,4CI3). b). Thalidomide. c). Lenalidomide. d). Pomalidomide. e). The Lenalidomide-CRBN interaction mode (PDB: 4CI1). f). The atomic level of Thalidomide-CRBN interaction (PDB: 4CI1).

made a significant discovery by identifying a new target of lenalidomide: CK1α (casein kinase 1α). Notably, CK1α cannot be degraded by thalidomide or pomalidomide (Table 1). This finding suggested, for the first time, that target degradation can be achieved by modifying the structure of molecular glues, opening new possibilities for tailoring these compounds for specific therapeutic applications [17].

Table 1. Substrate specificity and indications of IMiDs compounds.

Molecular Glue	E3 Ligase	Targets	Indications	Approved
Thalidomide	$CRL4^{CRBN}$	IKZF1/3	Multiple Myeloma	√
Lenalidomide	$CRL4^{CRBN}$	IKZF1/3, **CK1α**	MDS/Multiple myeloma/ lymphomas	√
Pomalidomide	$CRL4^{CRBN}$	IKZF1/3	Multiple myeloma/Kaposi sarcoma	√

The remarkable success of immunomodulatory imide drugs (IMiDs) like thalidomide, lenalidomide, and pomalidomide inspired extensive efforts in both academic and industrial research to develop novel IMiD analogues. In 2015, Celgene reported a significant breakthrough with a new compound, CC-122 [18] (Figure 2). CC-122 was engineered by maintaining the glutarimide ring, which is a characteristic feature of IMiDs, while altering the phthalimide part to include 2-methylquinazolinone. CC-122 more potently degraded transcription factors IKZF1/3 in diffuse large B-cell lymphoma (DLBCL) and T cells in vitro, in vivo, and in patients. Encouragingly, CC-122 showed broad cytotoxic activity in DLBCL as well as

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immunomodulatory activity. CC-122 was the first published molecular glue degrader with a different core structure rather than phthaloyl ring and offered us more explorable space about molecular glue design. In 2021, Ebert[18b] group revealed that CC-122 can also induce ZMYM2 and ZNF91 for degradation at the same time and demonstrated its potential application for treating hematologic malignancies. These results showed that other disease-causing protein targets could be targeted and degraded by modifying the chemical structure of IMIDs. In 2016, Celgene disclosed a new cereblon modulator CC-885[19] by modifying the phthalimide core structure with ureido-connected phenyl group (Figure 2). CC-885 was designed with a different strategy by keeping the phthaloyl core structure. Upon binding with CRBN, the GSPT1 was recruited by CC-885 to be ubiquitinated and degraded through proteasome system. Interestingly, GSPT1 does not have the common structure feature as previously reported C2H2 zinc finger [20] like IKZF1/3, which implies again that the CRBN induced degradable targets are not limited to C2H2 zinc finger proteins. Further studies revealed that GSPT1 and IKZF1 were not only the targets of CC-885. In 2020, Zhao and Tan group[21a, c] proved that CC-885 could also induce PLK1 and BNIP3L degradation, while Fang group disclosed that CDK4 was also a direct target of CC-885. The preceding research suggests that by adjusting the chemical structures of IMIDs, it is possible to enlist a greater number of innovative targets, albeit with potential concerns regarding target selectivity.

In 2018, Hansen team from Celgene [22] studied structure-activity relationships of CC-885 analogues in order to increase the target selectivity of GSPT1 deradation. They developed an in-house QSAR mode to predict SAR based on CC-885 structure. In this work, the author disclosed oxoisoindoline or dioxoisoindoline scaffolds and terminal groups played major role on achieving selectivity between GSPT1 and IKZF3. This work successfully also implied that chemical modification on the molecular glue could adjust target protein selectivity. Obviously, CC-885 encountered many problems in clinical developments due to unmanageable off-target toxicities associated with the degradation of multiple neosubstrates, including GSPT1, CK1α, HBS1L, IKZF1 (Ikaros) and IKZF3 (Aiolos). Moreover, although the degradation of GSPT1 is required for the anti-AML activity of CC-885, the missing mechanistic link between GSPT1 degradation and the subsequent anti-AML effect further hindered the development of CC-885. To solve the above problems, Lu team [23] from Bristol-Myers Squibb optimized the structure of CC-885 and prepared CC-90009 by replacing the terminal aniline group with 1-chloro-4-(difluoromethyl)benzene (Figure 2). Ternary crystal structural and degradation study disclosed partly the molecular mechanism that CC-90009 could selectively and potently target GSPT1 for ubiquitination and proteasomal degradation. In 2020, Gray group[23c] systematically explored the impact of small motifs (like −NH2, urea, or aniline) onto the IMiDs scaffold on protein-protein complementarity between CRBN and the targeted neo-substrate. Comparing with CC-885, the ZXH-1-161[23c] formed a new hydrogen bond between nitrogen (Labeled in red in figure 2) and Lys628 of GSPT1, which contributed to the high selectivity. These results implied that the modification of terminal group of molecule glue could adjust the target selectivity by forming specific interaction with target protein. In the meanwhile, in 2022, Monte Rosa developed their GSPT1 degrader (MRT2359) by changing the terminal group at C2 site of IMIDs core structure, and MRT2359 have been advanced into clinical experiment[23d] to treat solid tumors, including non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), high-grade neuroendocrine cancer of any primary site, diffuse large B-cell lymphoma (DLBCL), and tumors with L-MYC or N-MYC amplification.

Using strategy of ligand-based drug design, Chamberlain team from Celgene continued to publish novel IMIDs analogues. In 2018, CC-220[24a] as a new CRBN modulator that is more potent to degrade IKZF1/3 was reported (Figure 2). Comparing with previously reported molecules, CC-220 adopted morpholine as terminal group connected with phenyl group. Meanwhile, CC-220[24] exhibited good antitumor and immunostimulatory activities in lenalidomide- and pomalidomide- resistant multiple myeloma cells with dysregulated CRBN because of increased binding affinity with CRBN and more potent degradation of IKZF1/3. In 2020, Hansen team from Celgene discovered a new molecular glue[25] (CC-92480) through a phenotypic screen of their CRBN modulator library for the treatment of patients with relapsed or refractory multiple myeloma (RRMM). CC-92480 showed the enhanced efficiency to drive the formation of the protein-protein interaction between IKZF3 and cereblon compared with lenalidomide and pomalidomide, inducing a unique and rapid degradation profile of target. Although CC-92480 was discovered via phenotypical screening experiment, the structure of CC-92480 extended longer chain with phenyl group based on CC-220. Both CC-220 and CC-92480 have moved into clinical experiments, which also offered the examples of improving potency by modifying terminal group at the same core structure.

In addition to reported targets such as IKZF1/3, CK1α, GSPT1 etc., discovery of more CRBN neosubstrates hijacked by IMIDs analogues is becoming more urgent. In 2020, Thompson team[26] in Bristol Myers Squibb reported two new CRBN modulators

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(CC-3060 and CC-647, Figure 2) which can induce ZBTB16, a C2H2 ZnF transcription factor, for ubiquitination and degradation by CRBN E3 ligase and provided a potential therapeutic treatment for acute promyelocytic leukemia. Interestingly, CC-3060 and CC-647 required different degron motifs on ZBTN16: ZnF1 was necessary for CC-3060 induced degradation of ZBTB16 while ZnF3 was indispensable for CC-647. These` results implied that the residues of target proteins interacting with CRBN

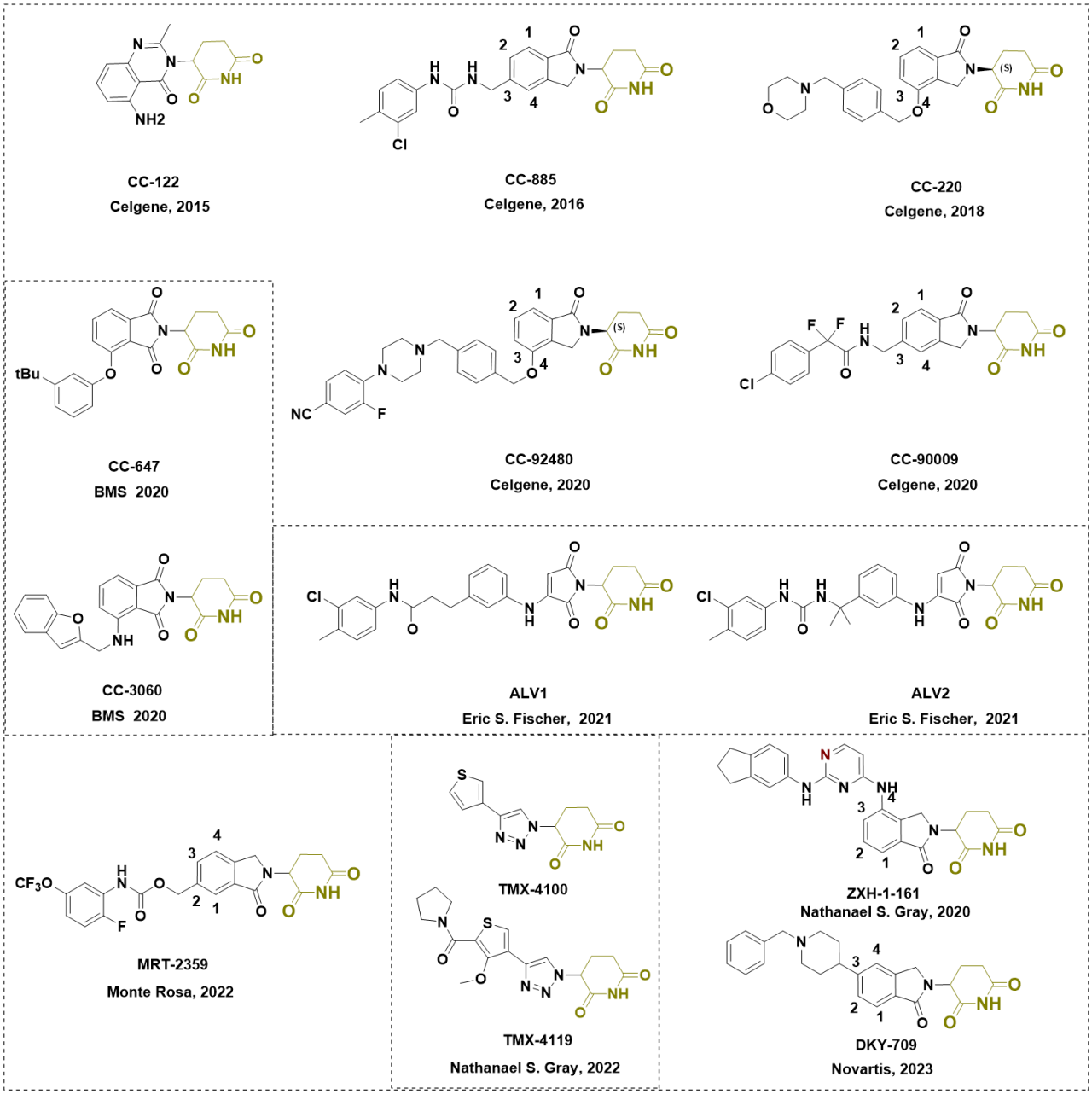

Figure 2. Structures of CRBN modulators.

and molecular glue both play an important role for target recruitment. In 2021, Gray and Fischer group[27] predicted IKZF2 was a potentially degradable target when they carefully analyzed the amino acid sequence of Ikaros proteins family. The presence of a glutamine residue in the second zinc-finger domain in IkZF1/3 enabled imide-induced degradation, but histidine residues contained in IKZF4/5 blocked imide-induced degradation. Meanwhile, they discovered that CC-885 can induce minimal degradation of IKZF1(Q146H) mutant by forcing weak interactions between CRBN and IKZF1(Q146H), while the IKZF1(Q146A) mutant rescued CC-885 induced degradation of IKZF1. These results suggested steric hindrance and electronic effect of histidine were the critical factor for the development of IKZF2 degraders. To adapt to the molecular environment formed by histidine, more flexible CRBN-binding core (pyrrole-2,5-dione) was used to replace the rigid phthalimide core to give ALV1 and ALV2 (Figure 2), which showed good capacity to degrade IKZF2. ALV2 has higher selectivity toward IKZF2 based on preliminary results. With a different approach, to adapt to the molecular circumstance of histidine, Solomon[28] and his team chose to modify the terminal group at C3 of IMIDs core and discovered DKY709(Figure 2). Clearly, modifying core structure or terminal group at different site of the backbone could recruit the same target. In 2022, Gray[29] designed a series of molecules using unique triazole as core structure to selectively degrade PDE6D or CK1a. This work

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showed the process of converting one target to another by reprogramming the core structure and terminal group. Meanwhile, it proved the possibility of modification at glutamide ring for CRBN modulators to achieve target selectivity. All these published works have paved a way to obtain more potent and selective CRBN based molecular glues by modification of molecular structure at the core and terminal group. However, there is still not a clear rule to guide us to rationally design a molecular glue toward to specific target.

Crystal Structures of Ternary Complex: CRBN-Molecular Glue-Target Protein

From those molecular glues and degradable targets, scientists have realized that all degradable targets shared a common character: they all contained a degron named β-hairpin-loop including glycine[16,17,19,20,32]. This character served as a powerful guidance to choose potentially accessible disease-causing protein targets for developing molecular glues. However, rational design of molecular glue for specific target is still unapproachable since how the minor adjustment on molecule structure resulting in target expansion is still unclear. To better understand the PPI in the presence of molecular glue on the molecular level, researchers made great efforts to solve the crystal structures of CRBN-IMIDs-Target ternary complex to shed light on the details of molecular interaction and potentially to guide the rational design with the better understanding of PPI.
In 2016, Thomä group[17a] successfully obtained a crystal structure of DDB1-CRBN bound to lenalidomide and CK1α (Figure 3). This crystal structure clearly disclosed how the lenalidomide induced the interaction between CRBN and CK1α: CRBN and lenalidomide jointly provided the binding interface for β-hairpin-loop of CK1α located in the C-terminal domain of CRBN (Figure 3). Multiple interactions (Figure 3b) between CRBN and CK1α were formed beyond original interaction between lenalidomide and CRBN (Figure 1). Three amino acids (N351, H357 and W400) around the IMIDs binding pocket of CRBN generated hydrogen bonds with I37, T38 and N39 from the β-hairpin-loop of CK1α respectively. In addition, the interface between CRBN and CK1α out of molecular glue binding site could also form interactions which might contribute to the

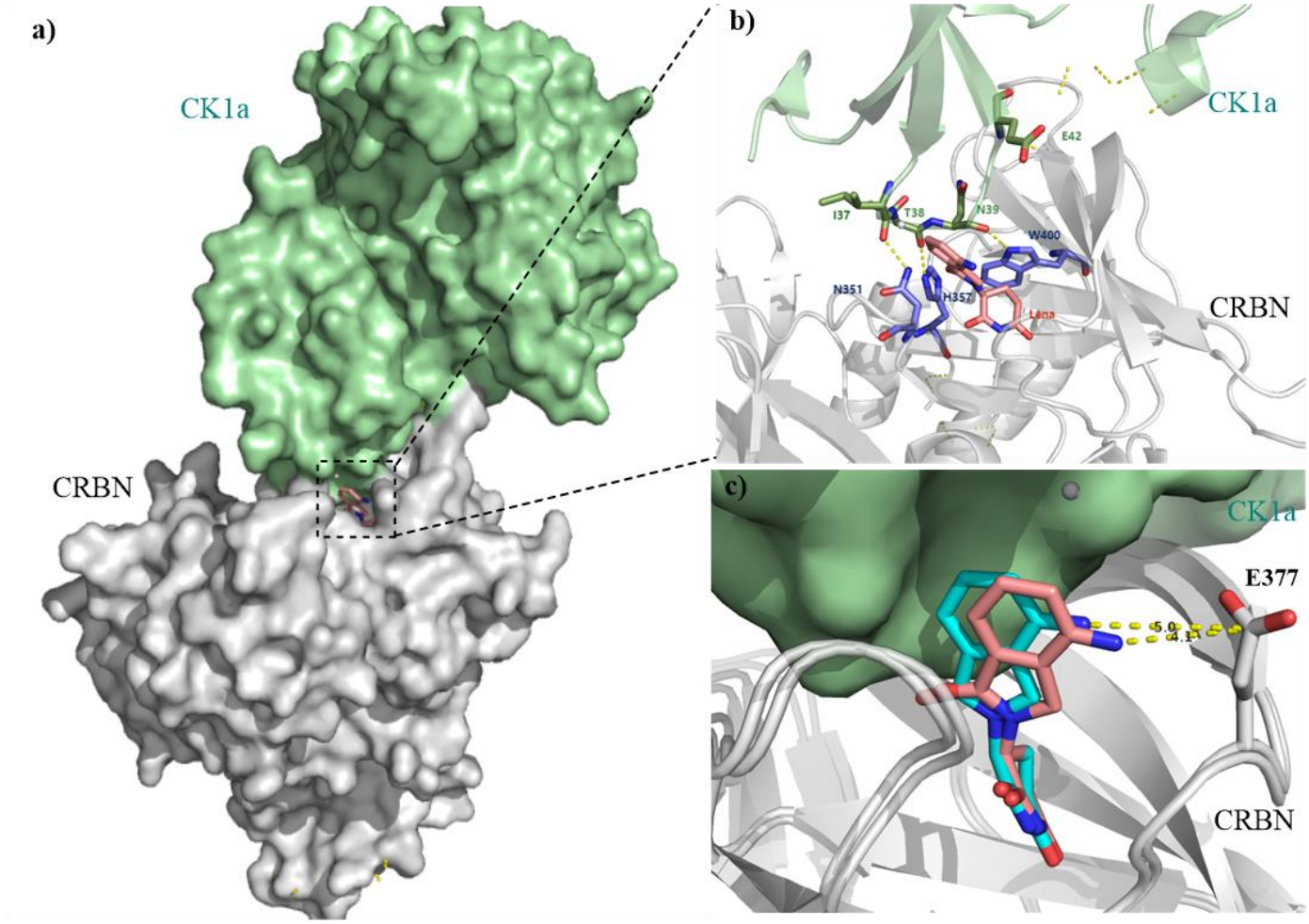

Figure 3. CK1α interacts with CRBN and lenalidomide. a). The ternary crystal structure of CRBN-Lena-CK1α (PDB: 5FQD). b). The interaction mode of CRBN-Lenalidomide-CK1α. c). Lenalidomide shifts toward E377 before and after binding with CK1α (Light Brown_PDB:5FQD, Cyan_PDB: 4CI2).

formation of ternary complex (Figure 3b). Meanwhile, by comparing the conformation of lenalidomide on the CRBN surface before and after binding with CK1α, Lenalidomide experienced slight shift toward to E377 of CRBN (distance shortened from

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5.0Å to 4.1Å) after binding with CK1α. This crystal structure clearly showed the dynamic picture of how lenalidomide induced the formation of the ternary complex.

In 2018, Thomä group reported ternary complex crystal structures of CRBN-Poma-IKZF1 and CRBN-Poma- ZNF692[20](Figure 4). Interestingly, the crystal structure of CRBN-Poma-IKZF1 showed that CRBN adopted an open conformation, with its N- and C-terminal domains separated (Figure 4a, b, c) while CRBN in CRBN-Poma-ZNF692 complex was in a close conformation. These two different conformations of CRBN disclosed a possible conformational transformation induced by IMIDs. To explain this phenomenon, Glander[30] and his team recently reported that molecular glues are allosteric regulators of CRBN conformation. Based on cryo-EM analyses of the DDB1-CRBN apo-complex in the presence or absence of molecular glues, they found that the binding with molecular glues at Thalidomide binding domain (TBD) is necessary to complete the transformation from open to close conformation for CRBN. This work showed that molecular glue inducing target protein degradation went through a dynamic conformational transformation of CRBN from open to close. This implied that the ability of inducing CRBN from open to close conformation may relate to degradation of protein of interest. The binding mode of pomalidomide in ternary complex of CRBN-Poma-IKZF1 and CRBN-Poma-ZNF692 (Figure 4d, e) depicted the features of how pomalidomide hijacked CRBN to bind with IKZF1 or ZNF692. Pomalidomide stayed in the tri-tryptophan hydrophobic pocket in the TBD domain of CRBN and formed hydrogen bonds with residues E377, H378 and W380 of CRBN. Meanwhile, when IKZF1 or ZNF692 was recruited to CRBN, the degron motif: β-hairpin loop was positioned at binding site of pomalidomide and new interaction among the three unites of ternary complex formed subsequently. Although the amino acid sequence is different in the β-hairpin-loop of IKZF1/ZNF692, the interaction does not vary too much. Besides van der Waals contact and hydrophobic interaction between pomalidomide with IKZF1 or ZNF692, new hydrogen bonds between IKZF1/ZNF692 and CRBN were also achieved: H353 and W400 from CRBN acted as hydrogen bond donor while the carbonyl group from β-hairpin loop of IKZF1/ZNF692 played the role of hydrogen bond acceptor within 3.5Å. However, for different targets, the conformation of residues on the surface of CRBN experienced slight transformation, especially for residues taking part in mutual interaction like E377 (Figure 4d, e). This result seemed to suggest us that the residues of target protein and CRBN in the interface could accommodate accordingly to form the final ternary complex in the presence of small molecule glue.

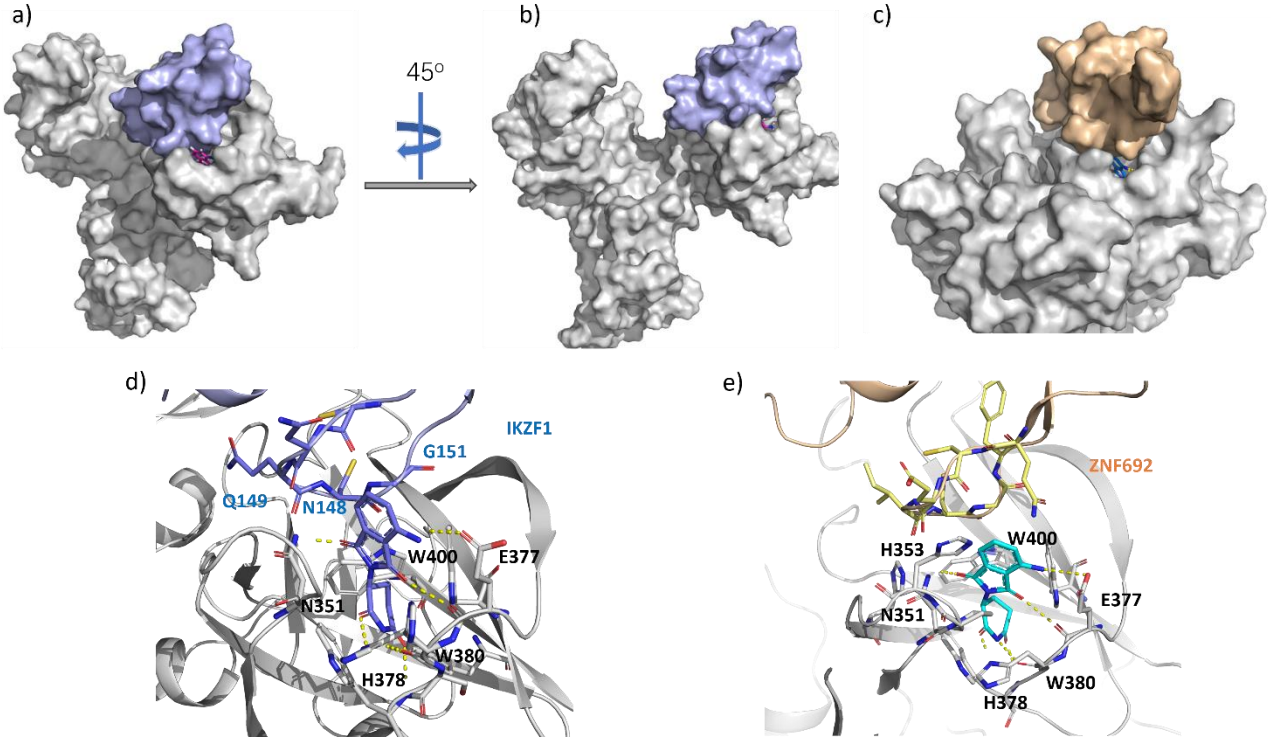

Figure 4. Interaction between IKZF1/ZNF692 and binary complex (CRBN and pomalidomide). a). The ternary crystal structure of CRBN-Poma-IKZF1 (PDB:6H0F). b) the ternary crystal structure of CRBN-Poma-IKZF1 after rotating 45o. c). The ternary crystal structure of CRBN-Poma-ZNF692(PDB:6H0G). d). Molecular interaction mode in pomalidomide-binding site of CRBN-Poma-IKZF1. e). Molecular interaction mode in pomalidomide-binding site of CRBN-Poma-ZNF692.

In 2016 and 2020, the ternary complex crystal structures of GSPT1_CC-885_CRBN [19] and GSPT1_CC-90009_CRBN [23a] were solved by Chamberlain and Lu teams respectively. As the crystal structures showed, the β-hairpin loop of GSPT1 positioned on the IMIDs binding surface induced by CC-885 and CC-90009. The conformation of GSPT1 did not experience changes

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among the two crystal structures (Figure 5a). However, after replacing the amide group with difluoromethane, the terminal phenyl group of CC-90009 experienced slight switch toward the hydrophobic region (Figure 5e) of GSPT1 in the ternary crystal structure (Figure 5b). These two crystal structures disclosed that CC-885 and CC-90009 stayed in the tri-tryptophan hydrophobic pocket in the TBD domain of CRBN (Figure 5a,5b), forming hydrogen bonds between the glutarimide ring and residues W380, H378, E377 of CRBN (Figure 5c,5d). In the meantime, both CC-885 and CC-90009 induced interaction between CRBN and GSPT1 by forming hydrogen bonds. The carbonyl group of K571, K572 and S573 from backbone of the β-hairpin loop of GSPT1 acted as a hydrogen bond receptor while W400 and H357 of CRBN played a role of hydrogen bond donor. It is worth mentioning that the residues from GSPT1 and CRBN out of molecular glue binding region may also form interaction: Q390 and E576 formed hydrogen bond in crystal structure of GSPT1_CC-885_CRBN (Figure 5c), and R613 and D149 formed hydrogen bond in the crystal structure of GSPT1_CC-90009_ CRBN (Figure 5d). In the crystal structure of GSPT1_CC-885_CRBN, a water molecular was also involved in the interaction between GSPT1 and CC-885 (Figure

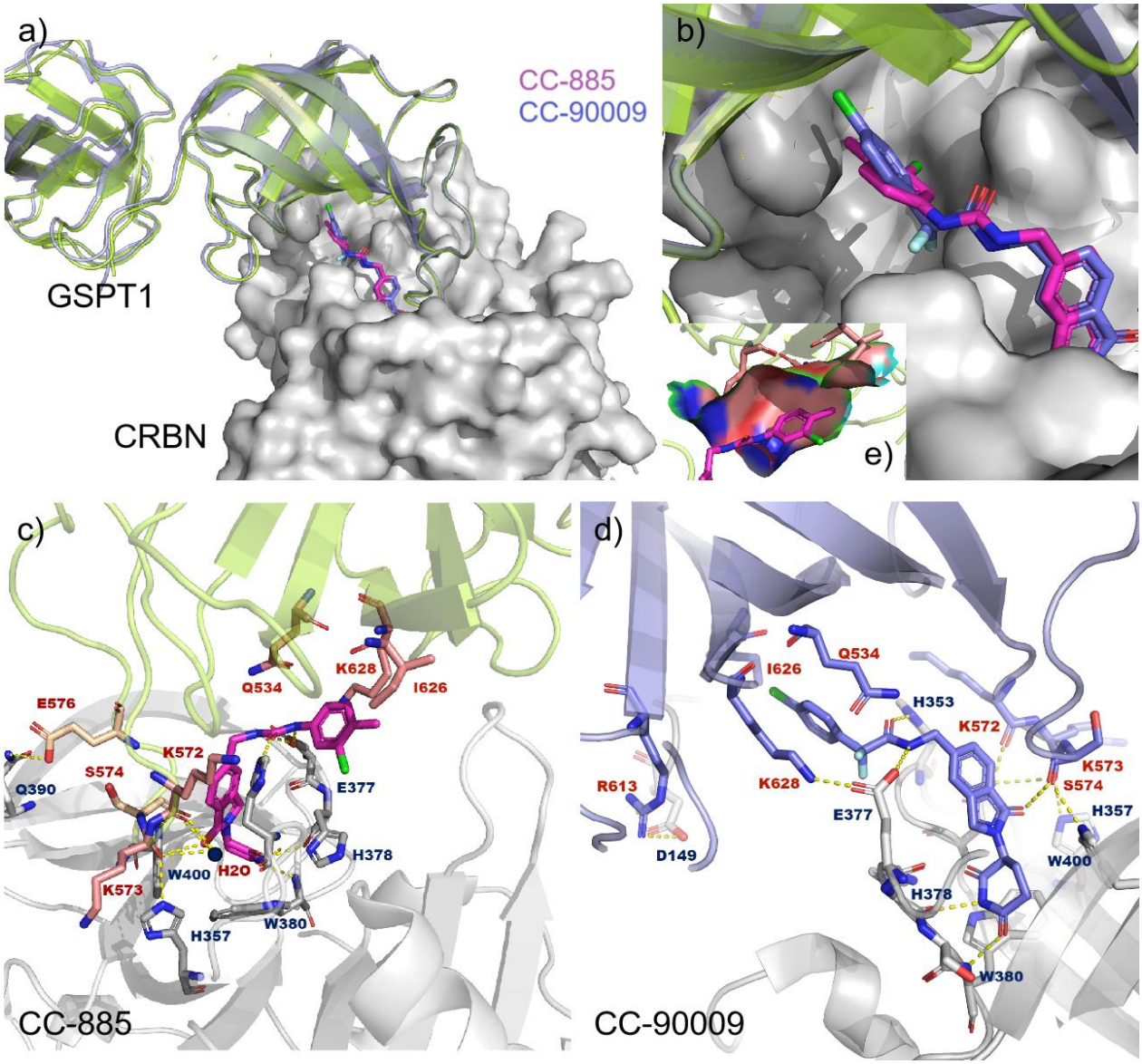

Figure 5. Crystal structure of CRBN_CC-885orCC-90009_GSPT1.a) the alignment of ternary crystal structure of CRBN_CC-885_GSPT1 (PDB:65HXB) and CRBN_CC-90009_GSPT1(PDB:6XK9) . b). The enlargement of crystal structure of CC-885 and CC-90009 in ternary complex crystal structure. c). The interaction mode of CRBN_CC-885_GSPT1. d). The interaction mode of CRBN_CC-90009_GSPT1 e). The enlargement of interaction between terminal phenyl group of CC-885 and GSPT1

5c). Moreover, CC-885 and CC-90009 induced new interactions with GSPT1 and CRBN with the extended urea or the amide connected to phenyl group. First, the amide group of CC-885 and CC-90009 formed hydrogen bonds with E377 and H378 of CRBN. The terminal phenyl group of CC-885 and CC-90009 sits in the hydrophobic surface formed by I626, Q628 and Q534 (Figure 5c,5d,5e) which may also contribute to the formation of the ternary complex. In 2021, the Fischer group successfully obtained a ternary crystal structure of IKZF2_ALV1_CRBN (Figure 6a, 6b)[27]. Like other reported crystal structures, the β-

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hairpin loop of IKZF2 was positioned at the molecular glue binding site on the surface of CRBN hijacked by ALV1. Although the core structure of ALV1 was changed compared to that of IMIDs, the binding mode was consistent: the glutarimide ring stayed in the tri-tryptophan pocket of CRBN and formed hydrogen bonds with residues W380, H378 and E377 of CRBN. Meanwhile, W400 and H357 of CRBN formed hydrogen bond with the carbonyl group (N143, C145) on the backbone of the β-hairpin loop within the distance of 3.5Å. These interactions were also in the targets such as IKZF1, ZNF692, CK1a and GSPT1. However, being different from other targets, N351 of CRBN took part in the interaction with IKZF2 by forming hydrogen bonds with N143. On the other hands, N351 also formed a hydrogen bond with the carbonyl group of ALV1. Meanwhile, ALV1 showed that GSPT1

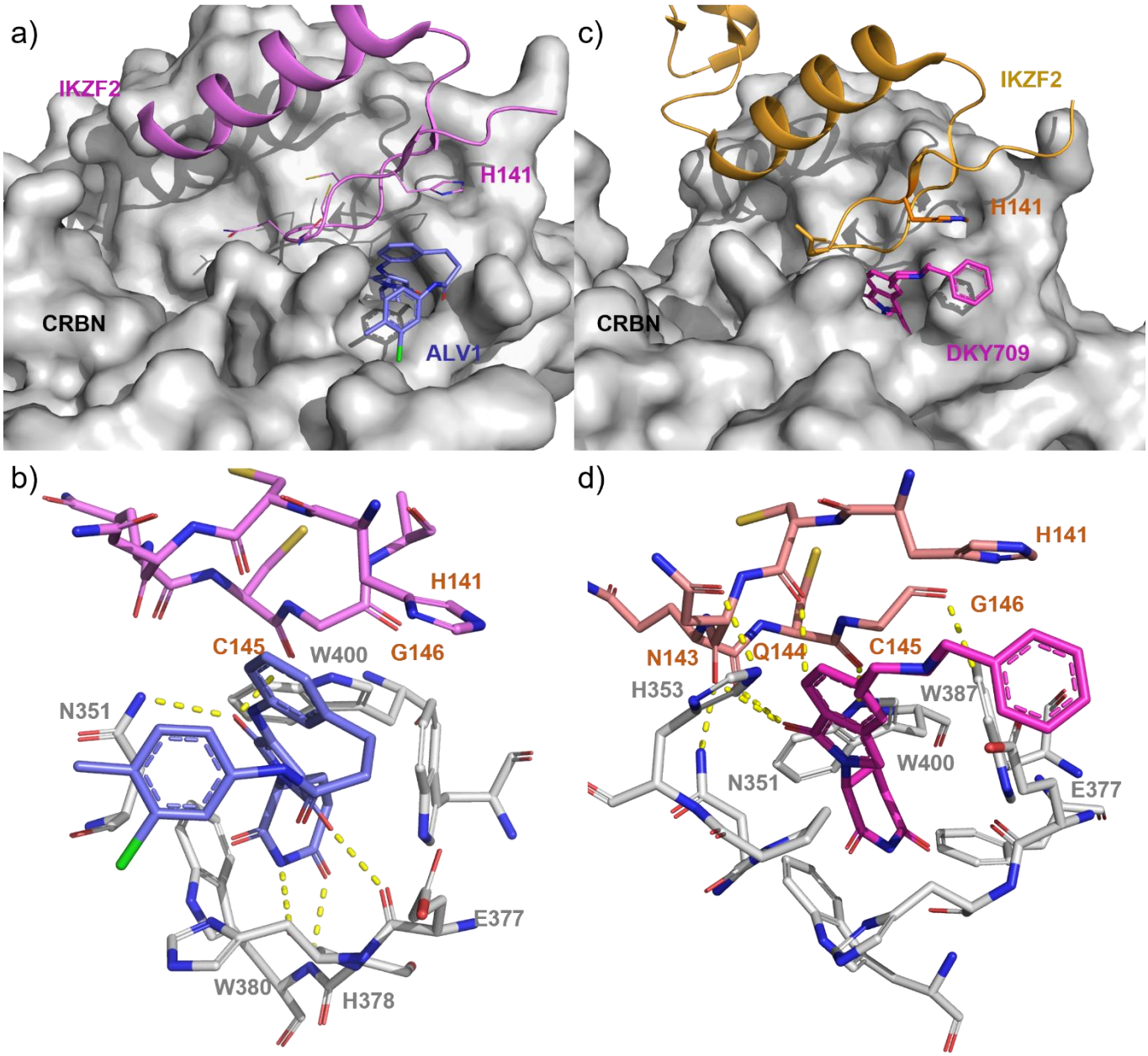

Figure 6. a) Crystal structure of IKZF2_ALV1_CRBN(**7LPS**). b) Interaction analysis of ternary complex between IKZF2, ALV1 and CRBN

degradation was avoided (Figure 6b). The Fischer group compared the ternary crystal structure between GSPT1 and IKZF2 and proposed that the tail of ALV1 stayed at a noticeably different location compared to the common 3-chloro-4-methylphenyl tail of CC-885, which would clash with V536 of GSPT1. The hydrophobic ethylbenzene moiety of ALV1, as compared to the urea moiety of CC-885, was in close proximity to GSPT1 Q534 with potential for steric clash, providing a structural rationale for the inability of ALV1 to degrade GSPT1.[27] Obviously, residues of β-hairpin-loop greatly affect the formation of ternary complex based on published results. On other hand, Solomon team[28] discovered another IKZF2 degrader(DKY709) that featured the same core structure with CC-885. From the crystal structure (Figure 6c, 6d), DKY709 adopted a different gesture on binding with CRBN: the phenyl tail of DKY709 turned to E377 side while ALV1 turned to H353 side (Figure 6a, c, e). Moreover, the interaction strength induced by ALV1 and DKY709 is very different. Besides inducing the

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hydrogen bond interaction between IKZF2 and CRBN, DYK709 formed many interactions with the carbonyl group (N143, Q144 and C145) from β-hairpin loop. The carbonyl group of DKY709 induced tri-interaction with N143(IKZF2), N351(CRBN) while the W387 of CRBN formed hydrogen bond with G146 of IKZF2. They found that ZF2-3 showed greater than 22-fold affinity of binding to DDB1-CRBN: DKY709 complex compared to ZF-2 alone. This suggests that ZF3 also contributes to binding during the formation of ternary complex. Although DKY709 and ALV1 both can induce formation of ternary complex between IKZF2 and CRBN, the interaction mode and strength are obviously different, which exhibits the tolerability toward chemical structure alteration.

From these published results of degradable proteins and ternary crystal structures, the β-hairpin loop with glycine at equivalent site is the common structural character. In addition, this β-hairpin loop structure is positioned on the molecular glue binding site in same gesture and the conservative glycine in β-hairpin loop interact with Valine(V388) of CRBN. The carbonyl group in the β-hairpin loop will take part in the interaction with other components in ternary complex. The residues of each β-hairpin loop might be responsible for degradation selectivity.

Outlooks

Undeniably, molecular glues have received increasing attention from academia and industrial researchers recently. Besides molecular glue exhibited lower molecular weight and high druggability, the advantage of pocket-independency imparts molecular glue the possibility to access to targets which are regarded as undruggable (account for more than 80% of proteins). The success of IMIDs compounds in treating multiple myeloma and lymphomas have showed the great potential of molecular glues as useful therapeutics. Along with increasingly discovery of creative molecular glues toward different protein of interest, we have obtained increasing knowledge on the mechanism of actions of the molecular glues. These learnings in turn could help the optimization of new molecular glue degraders.

In the aspect of molecular glue, it consists of three parts: glutarimide, core structure and balancing groups (Figure 7a)[32]. Certainly, glutarimide is critical for binding to CRBN in the tri-tryptophan pocket. The core structure mainly stayed in the pocket formed by CRBN and β-hairpin-loop of target protein and involved in the interaction between β-hairpin-loop and CRBN. Core structure modification of molecular glue could be the first major strategy to control target protein degradation and target selectivity. For example, in the structure and activity study of CC-90009[22], the modification of core structure effectively influences the degradation potency and selectivity. In addition, Sawasaki[33] and his team reported modification with different functional group at different position of core structure could decide target protein selectivity. On the other hand, the extended group (balancing group, Figure 7a)[23a,b] stretch out from the door formed by residue E377 and N353. This balancing group can ether improve the selectivity or activity by forming additional interactions like CC-885(Figure 5). Moreover, core structure and balancing group together could be considered as the anchor of adjusting Pharmacokinetic property.

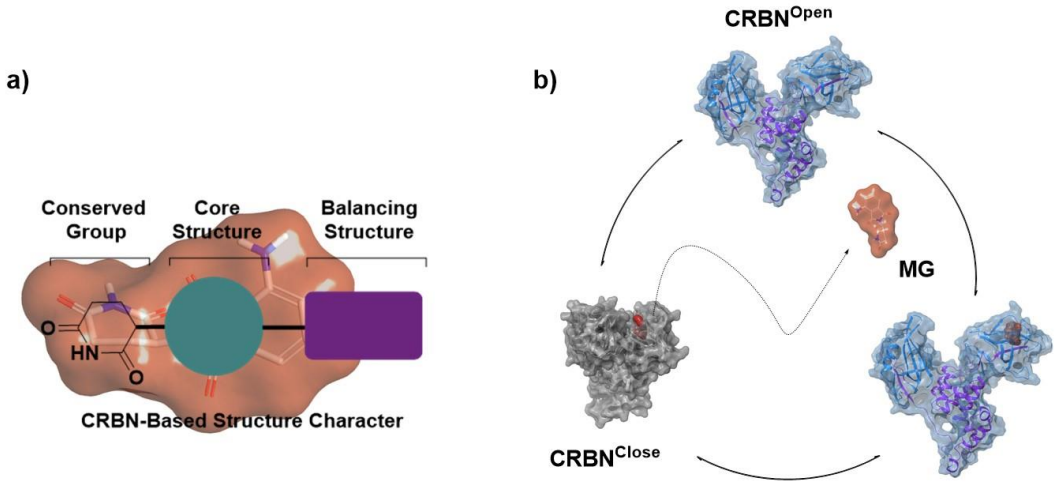

Figure 7. a) Model of molecular glue structure for CRBN). b) Dynamic process of molecular glue induced ternary complex formation

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In the process of recruiting target proteins, molecular glue first bind to the binding domain of CRBN and will quickly induce the conformation alteration from open to close[30] form (Figure 7b). This procedure suggests that binding affinity between CRBN-molecular glue and conformation inducing ability from open to close can all affect the degradation capacity. Then the binary complex recruits the protein of interest for ubiquitination and degradation, which is another limitation step for degradation. These mechanism gives more inspiring supports for molecular glue discovery and design.

For an unknown potential targets, successful rational design of the corresponding molecular glues for specific target protein has not been achieved so far. Based on the successful application of reported crystal structure of ternary complex on optimization of molecular glues[22], it may be a possible and powerful strategy to use computational tools to predict the crystal structures of binary complexes between targets and CRBN to form the "pocket" via protein-protein docking. The predicted pocket formed by protein-protein interaction (PPI) may also guide the design of molecular glues with structure based drug design[31] strategy, and the predicted pocket could also be used to proceed virtual screening, especially when the precision of in-silico predicted PPI binary complex satisfy the requirement. Meanwhile, the arise and application of machine learning in drug developments may also guide the design of molecular glues and the selection/identification of specific targets along the accumulation of data of molecular glue[34].

In summary, the arise of CRBN-based molecular glue offers a new small molecular drug modality to treat disease. The reported successful cases also demonstrated the huge potential in targeting various disease-causing proteins, especially for undruggable target proteins like scaffold proteins, transcription factors by modification of IMIDs structure. It will be a great advance if molecular glue compounds could be rationally designed toward specific disease-causing protein. This requires a concerted efforts from all the target protein degradation community.

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