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Title: Recognition of substrate degrons by E3 ubiquitin ligases and modulation by small-molecule mimicry strategies

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Highlights

- E3 ligases recruit substrates for proteasomal degradation by recognition of degrons
- Crystal structures reveal the structural basis and mechanism of degron recognition
- Small-molecule degron mimetics can prevent or re-direct substrate recognition
- **Small-molecule inducible degrons and PROTACs enable targeted protein degradation**

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Abstract

The ubiquitin-proteasome system is a master regulator of protein homeostasis, by which proteins are initially targeted for poly-ubiquitination by E3 ligases and then degraded into short peptides by the proteasome. Nature evolved diverse peptidic motifs, termed degrons, to signal substrates for degradation. We discuss degrons of the N-end rule pathway and also degrons characterized by post-translational modifications, including phosphorylation and hydroxylation. In each case we detail the structural basis of E3 ligase:degron recognition and small-molecule mimicry approaches that disrupt those protein-protein interactions. We present as well genetic and chemical technologies that enable targeted degradation of proteins of interest, **namely small-molecule dependent inducible degrons and chemical degraders, e.g. proteolysis-targeting chimeras (PROTACs).**

Introduction

The Nobel Prize in Chemistry 2004 was awarded jointly to Aaron Ciechanover, Avram Hershko, and Irwin Rose “for the discovery of ubiquitin-mediated protein degradation”. Since then, many discoveries have paved the way to a better mechanistic and structural understanding of the protein degradation machinery and have enabled its purposeful modulation and hijacking.

The ubiquitin-proteasome system (UPS) is a complex cellular pathway by which proteins are first ubiquitinated and subsequently unfolded and proteolyzed by the proteasome. This process has direct implications primarily on regulating protein homeostasis and, depending on the context, can impact many cellular signaling processes, including cell cycle, DNA repair, apoptosis, inflammation, transcription regulation, stress response, and protein quality control (PQC) [1]. Three main enzymes are responsible for the specific targeting of proteins for degradation: E1-activating enzymes, which activate ubiquitin (Ub) in an ATP-dependent manner; E2-conjugating enzymes, to which the activated Ub is covalently attached to yield an E2~Ub thioester intermediate; and E3 ubiquitin ligases, which catalyze the transfer of Ub from the E2 enzyme to form an isopeptide bond with a lysine residue on the protein substrate (mono-ubiquitination or priming) or its covalently attached Ub (poly-ubiquitination) [2]. To act as catalyst in the process, E3 ligases typically recruit specific target substrates for degradation by recognition of peptidic segments termed ‘degrons’ as characterizing signaling markers [3]. The structural determinants within the degron and the E3 ubiquitin ligase that confer substrate specificity and dictate protein recognition and fate are of utmost importance to elucidate and be able to manipulate proteasome-mediated degradation and are the focus of this review. Recognition of structural protein domains or specific consensus sequences, *e.g.* in the case of D-box and KEN-box recognition by anaphase-promoting complex/cyclosome (APC/C)[4] will not be covered here.

Nature has evolved diverse mechanisms to regulate protein homeostasis. For example, orchestrated autophagy of misfolded or damaged proteins is intimately linked to the UPS through the PQC pathway [5]. Those substrates can be targeted for degradation in different ways, including exposure of hydrophobic degrons that would be otherwise buried inside the protein or post-translational polyglycosylation of Asn residues. In the latter case, misfolded proteins are signaled for endoplasmic reticulum-associated protein degradation (ERAD) [6]. Failure to degrade misfolded proteins, consequently favoring their aggregation and eventual

collapse, has a major impact in the development of neurological diseases [7, 8]. Other post-translational modifications (PTMs) apart from Asn glycosylation, such as phosphorylation of Ser, Tyr, and Thr residues, hydroxylation of Pro, and acetylation of Lys and their interplay contribute as well to determining a protein's fate [9]. For example, acetylation competes with Lys ubiquitination and can prevent target degradation [10]. In other cases, the E3 ligase itself post-translationally modifies the substrate upon engagement, which in turn allosterically initiates the ubiquitination cascade of the target protein [11].

In this review we first briefly discuss degradation of proteolytic cleavage products by the N-end rule pathway. We next examine recruitment to E3 ligases of substrates marked for degradation by means of recognition of specific PTMs, namely phosphorylation and hydroxylation. We conclude exploring prominent small molecules from both natural and unnatural origin capable of modulating or even *de novo* re-directing substrate specificity of E3 ligases. In each case we describe related chemical biology tools for targeted protein degradation.

N-degrons

The pioneering observation of an apparent correlation between the presence of a free α -amino group in a protein and its ubiquitin-dependent degradation led to the formulation of the 'N-end rule', by which the *in vivo* half-life of a protein can be determined by the nature of its N-terminal amino acid, also termed 'N-degron' [12]. N-degrons are generated within the cell when specific residues are exposed at the N terminus by proteolytic cleavage. There are two classes of destabilizing N-degrons: positively charged amino acids (Arg, Lys, and His) are of type 1, and bulky hydrophobic ones (Phe, Trp, Tyr, Leu, and Ile) are of type 2 [13].

Conversely, other N-terminal amino acids such as Met and Cys confer stability against proteosomal degradation [14]. In eukaryotes, N-degrons are recognized by N-recognin, a UBR box motif present in E3 ligases that targets the substrate for ubiquitin-dependent proteosomal degradation [15]. For example, endoproteolytic cleavage of Scc1, a subunit of the cohesion complex in yeast, results in a type 1 Arg N-terminal fragment that is recognized and targeted for degradation by N-recognin UBR1. Notably, the fragment becomes lethal if accumulated [16].

The crystallographic structure of the UBR box of *S. cerevisiae* UBR1 in complex with type 1 N-degrons revealed that specific recognition is achieved by an intricate network of hydrogen-

bonds involving as well the amino acid in position 2 of the N-degron, which occupies an interfacial hydrophobic groove (Fig. 1a) [17]. Conversely, in type 2 N-degrons exquisite selectivity is accomplished by a highly conserved gatekeeper Tyr residue, which excludes Val but not the Ile, Leu, Phe, Tyr, and Trp degrons [15]. Interestingly, in bacteria, where Met instead of Tyr serves as gatekeeper residue, distinct selectivity is achieved by introducing steric clashes: bacterial UBR1 excludes Ile, Thr, and Val but not Leu, Trp, and Phe (Fig 1b and 1c) [13, 18, 19]. In eukaryotes, the default N-terminal amino acid is Met (*N*-formylmethionine in bacteria). The striking ability of N-recogin to discriminate Met from its structural cousin Leu with up to 1000-fold selectivity has been deeply investigated. Notably, only a rare, entropically unfavored Met rotamer can fit in the N-recogin cavity and avoid large van der Waals steric clashes with the surrounding residues. Moreover, this rotamer locates the Met's C ϵ in a chemically unfavorable environment [18].

Small molecules, *e.g.* *p*-Chloroamphetamine, are known to inhibit the N-end rule pathway by blocking a UBR recognition site [20]. However, broader applicability of such inhibitors to manipulate the cellular level of specific proteins is dramatically hampered by the lack of control on which substrate is downstream degraded. This limitation can be overcome by two distinct chemical biology approaches. In a first strategy, selective proteasome-mediated degradation of glutathione-S-transferase α 1 (GST- α 1) was achieved by linking a Boc-protected Arg (Boc₃-Arg) to a potent GST- α 1 covalent inhibitor [21]. A clear advantage of this technology is its intrinsic modularity with respect to which protein can be addressed. For example, use of a noncovalent inhibitor of dihydrofolate reductase (DHFR) conjugated to Boc₃-Arg led to rapid and robust DHFR degradation in cells. In contrast, linkage of the inhibitors to non-protected Arg rendered inactive degraders, indicating that Boc₃-Arg tagging works independently of the N-end rule pathway [21]. While the defined biological mechanism of Boc₃-Arg tagging is not fully understood, Long *et al.* used cycloheximide blocking to show that reduction of DHFR levels was due to induced degradation and not to translation inhibition [21]. Very recently it has been shown that Boc₃-Arg tagging localizes the target proteins directly to the 20S proteasome and stimulates its degradation without requiring ubiquitination [22]. A related approach called hydrophobic tagging (HyT) has been developed to append hydrophobic moieties to ligands and fusion proteins to induce targeted degradation [23, 24]. In a different approach, a Trojan horse genetic strategy was conceived by Taxis *et al.*, who developed tobacco etch virus (TEV) protease-mediated induction of protein instability (TIPI) [25]. TIPI is a method to genetically control the abundance of a

protein of interest (POI) by genetically inserting a dormant destabilizing N-degron. Upon expression of a site-specific protease, the dormant N-degron becomes exposed and triggers selective targeting of the POI by UBR and its proteasome-mediated degradation [25].

Phosphodegrons

Phosphorylation at one or several amino acids on **proteins is well known to direct formation of new protein-protein interactions (PPIs). The first protein module identified as a “reader” of phosphorylated protein modifications** was the Src homology 2 (SH2) domain, which belongs to the protein kinase family and recognizes exclusively phosphorylated Tyr (pTyr) [26]. Later on pTyr-, as well as pSer- and pThr-binding domains have been identified in other protein families and their crucial involvement in cell signaling and DNA damage response have become apparent [27].

Protein phosphorylation in regions so-called ‘phosphodegrons’ is also exploited for effective substrate recognition by E3 ligases and processive proteasome-mediated degradation [15]. A well-studied phosphodegron-binding system is the archetypical S-phase kinase-associated protein 1 (Skp1)-Cul1-F-box (SCF) Cullin RING ligase (CRL) (Fig. 2a), in which the variant F-box domain dictates substrate recognition. This family can be classified according to the presence of specific substrate recognition domains into FBWX, containing WD40 repeats, FBXL, presenting Leu-rich motifs, and the less characterized FBXO subclass [28]. For example, F-box WD40-containing protein 7 (FBW7) is the substrate recognition module of the cyclin-dependent kinase (CDK) regulator complex SCF^{FBW7}. Crystallographic studies of SCF^{FBW7} and its yeast ortholog, SCF^{Cdc4}, revealed that phosphodegron recognition in this system is driven by three primary features at the PPI interface: electrostatic interactions and a rich hydrogen-bond network that discriminate and exclusively trap the phosphorylated state of the target degron; hydrophobic patches that recognize two conserved hydrophobic residues in the phosphodegron; and positively charged residues that prompt suboptimal binding of basic phosphodegrons (Fig. 2b and 2c) [29, 30]. In yeast, SCF^{Cdc4} targets for poly-ubiquitination and proteasome-mediated degradation phosphorylated substrate inhibitor of CDK1 (SIC1), thereby enabling entry into the cellular S phase. Orlicky *et al.* carried out a screening of 50,000 small molecules to identify inhibitors of CDC4 that would prevent degradation of SCF^{Cdc4} targets. They identified an allosteric modulator of SCF^{Cdc4} that inhibits recruitment of pSIC1 by intercalating within the β -propeller of Cdc4, ~25Å away

from the phosphodegron recognition site of Cdc4 (Fig. 2d) [31]. In mammalian cells, SCF^{FBW7} recruits a number of important regulatory factors in cell growth and division pathways that function as proto-oncogenes in many cancers, such as cyclin E, MYC, and NOTCH, signaling them for ubiquitination and degradation [32]. Cancer-associated mutations in *Fbxw7* and in the genes encoding SCF^{FBW7} substrates can weaken binding affinities of the E3 ligase for its substrate degrons [32]. Small-molecule rescue, as opposed to disruption, of these PPIs could provide therapeutic benefit against prevalent mutant cancers. **Other examples of phosphodegron reader subunits in E3 CRLs include the Suppressor of Cytokine Signaling (SOCS) proteins and Cbl, each containing SH2 domains as substrate-recognition domain [33, 34].**

Rational design of small molecules that disrupt the recognition of phosphorylated targets usually relies on occupying the canonical phosphate-binding site of the reader protein. **This typically involves developing peptidomimetics, *i.e.* fragments of the native substrate that retain structural features of the molecular recognition motif while improving specific physicochemical properties. Alternative approaches involve identifying hits from screening compound libraries. A limitation of those methods is that resulting** molecules often lack selectivity amongst phosphodegron recognizers. For instance, the SH2-containing transcription factors signal transducer and activator of transcription (STAT) 5a and STAT5b have a sequence identity of 93% and recognize the same substrate peptide motifs, despite tissue-specific expression patterns and a number of non-redundant biological functions [35, 36]. The observation that catechol bisphosphate is a sub- μ M inhibitor of STAT5b with 35-fold selectivity over STAT5a motivated the development of a series of peptidomimetics using the catechol bisphosphate fragment as anchor [37]. The most potent compound, Stafib-1, has a K_i of 44 nM for STAT5b with over 50-fold selectivity over STAT5a and retains selectivity in tumor cells when formulated as a prodrug derivative [37]. Structural features that could shine light on the exquisite selectivity exhibited by Stafib-1 remain elusive.

Oxygen-dependent degrons (ODDs)

The modularity of the CRL architecture enables a dynamic and context-specific recruitment of substrate-binding proteins [38]. A notable case is the von Hippel-Lindau (VHL) protein, which forms part of an E3 ligase complex with the adaptor proteins Elongin (Elo) B and EloC, Cul2, and RBX1 (CRL2^{VHL}) (Fig. 3a). VHL recognizes and targets for degradation

hypoxia-inducible factor alpha (HIF- α) subunits, which are efficiently *trans*-4-prolyl hydroxylated (Hyp) under normal oxygen levels [39, 40]. In contrast, under hypoxia HIF- α subunits escape hydroxylation and recognition by VHL, are consequently stabilized inside cells, and drive transcriptional responses to hypoxia. Crystallographic studies revealed the structural basis for HIF-1 α binding by VHL, and elucidated the exquisite specificity for the recognition of the C⁴-*exo* conformation of Hyp (Fig. 3b) [41-43]. This mechanism of substrate recognition inspired the structure-guided fragment-based design of non-peptidic small-molecule Hyp derivatives that mimic binding of the natural substrate (Fig. 3c) [44-47]. By occupying the PPI interface of CRL2^{VHL}:HIF-1 α , these molecules could effectively displace HIF-1 α binding with nanomolar potency [47]. Further optimization of this class of inhibitors led to the discovery of VHL inhibitor VH298 as a novel potent, selective, and cell-active chemical probe of the VHL-HIF pathway [48]. VHL inhibitors have therapeutic potential in certain disease conditions where accumulation of HIF- α subunits and subsequent triggering of hypoxic response could prove beneficial [49].

Small-molecule dependent degrons

Methods to induce conditional and controlled degradation of POIs have substantial potential as both chemical biology and therapeutic tools. Interestingly, plants have evolved two analogous induced protein degradation mechanisms by phytohormones auxin and jasmonate as part of their signalosome [50, 51]. Transport inhibitor response 1 (TIR1) is the F-box substrate recognition subunit of a SCF^{TIR1} ubiquitin ligase (Fig. 4a), which targets transcriptional repressors known as Aux/IAA (indole-3-acetic acid) proteins for proteasomal degradation. By binding auxin, TIR1 increases affinity for its targets and triggers their rapid ubiquitination and proteasomal degradation [50, 52]. Crystal structures of Arabidopsis TIR1 in complex with auxin and an Aux/IAA degron peptide derived from the IAA7 protein elucidated the structural basis of how auxin binding directs TIR1:substrate interactions (Fig. 4b) [50].

Inspired by this natural mechanism, Nishimura *et al.* developed an auxin-inducible degron (AID) for the controlled degradation of proteins [53]. First applied to yeast, the method involves knock-in of the AID at either end of the POI, so that the fusion protein can be rapidly and efficiently depleted upon addition of auxin to the culture medium and conditional expression of the plant SCF^{TIR1} ubiquitin ligase [54]. The auxin degron technology has

proven its potential to study the biological function of proteins in higher eukaryotes. For example, it has been recently applied to induce rapid and conditional depletion of essential genes, for which knockouts or small-interfering RNAs are not suitable, in human and embryonic stem cells by introducing the AID-POI fusion using the CRISPR/Cas9-based method [54].

From a structural point of view, auxin and jasmonate act as “molecular glue” of specific PPIs, *i.e.* they stabilize the interaction of the substrate-binding domain of their respective E3 ligase and specific substrates [55]. Strikingly, phthalimide immunomodulatory drugs (IMiDs) thalidomide and its second-generation derivatives lenalidomide and pomalidomide act dually as molecular glues and PPI disruptors in humans by targeting the protein cereblon (CRBN) [56]. CRBN is the substrate-binding domain of the Rbx1-Cul4-DDB1-CRBN (CRL4^{CRBN}) E3 ubiquitin ligase (Fig. 4c). IMiD-binding by CRL4^{CRBN} prevents engagement of its endogenous substrate MEIS2; it also re-directs effective recruitment and CRBN-dependent degradation of the transcription factors Ikaros and Aiolos as well as Casein kinase 1 α (CK1 α) [57-60]. Additionally, lenalidomide derivative CC-885 was shown to induce recruitment and degradation of the translation termination factor GSPT1 [61]. These observations demonstrate that substrate selectivity of E3 ligases can be effectively modulated by binding of small molecules, which can act either as stabilizers or disruptors of specific E3 ligase:degron complexes. The structural basis of small-molecule induced recognition of CK1 α and GSPT1 by CRL4^{CRBN} revealed a molecular glue mechanism similar to auxin [61, 62]. Crystallographic data along with site-directed mutagenesis studies on a homology model of the Ikaros:CRBN complex further demonstrates that a hairpin-loop with low sequence homology but conserved topology serves as key structural degron for IMiD-induced CRBN recognition of substrates (Fig. 4d) [61].

PROTACs: Small-molecule directed protein degradation

Small molecules can be designed to recruit proteins into proximity to E3 ligases to induce target degradation. Proteolysis-targeting chimeras (PROTACs) are heterobifunctional molecules composed of a ligand for an E3 ligase and a ligand for a POI, connected by a linker [63]. PROTACs that hijack CRL2^{VHL} and CRL4^{CRBN} using derivatives of the VHL and CRBN ligands shown in Fig. 3 and 4 have proven very successful in inducing degradation of the epigenetic regulators BET bromodomain proteins (BRD2, BRD3 and BRD4) and the

estrogen-related receptor α (ERR α) in cells and *in vivo* [64-67]. Crucially, PROTACs can exhibit higher selectivity for protein degradation than one might anticipate based on the intrinsic binding selectivity of the warhead target ligand. For example, Zengerle *et al.* showed that VHL-targeting PROTACs based on the pan-BET inhibitor JQ1 induced preferential depletion of BRD4 in cells [64]. Lai *et al.* later also found that specific PROTACs engaging VHL or CRBN have distinct degradation preferences for their target kinases [68]. The substoichiometric catalytic modality of PROTAC's activity relieves the need to fully occupy a target binding site, aiding differential efficacy. Furthermore, the nature of the targeted E3 ligase [69], the chemical nature of the ligand and choice of derivatization points from the ligands, as well as possible cooperativity of ternary complex formation can all influence PROTAC's activity and play a role in enhancing target selectivity.

The large number of E3 ligases (> 600) encoded in the human genome [70] and the diversity and specificity of degron recognition motifs (reviewed recently in ref. [71]) provide numerous opportunities for PROTAC drug development. To date, only a handful of E3 ligases (including CRBN, VHL, MDM2 and IAP, Table 1) have been effectively hijacked by all small-molecules PROTACs using the respective E3 ligands. However, drug-like small-molecule ligands are beginning to emerge for more E3 targets (Table 1), suggesting other unexplored E3s may prove amenable to structure-based drug design.

PROTACs are an emerging technology that is attracting interest as chemical tool for target validation due to its simplicity and modularity. Recent improvements in efficacies and selectivity of PROTACs support development as new therapeutic modality [72]. However, structural and mechanistic details regarding PROTAC-induced complexes between E3 ligase and POI, and POI's processive ubiquitination remain to be elucidated.

Conclusions

We present here a selection of different degrons that E3 ligases recognize to specifically target substrates for proteasome-mediated degradation. The examples presented highlight how we are only beginning to scratch the surface of proteasome-mediated protein degradation, with more mechanisms of degron recognition likely to emerge in future. We anticipate that unraveling the overall structure and dynamics of E3 ligase:substrate complexation above and beyond epitope recognition for degron engagement will pave the way to a more detailed mechanistic understanding of processive ubiquitination. Beyond their

relevance to ubiquitin-specific mechanisms, the studies of E3 ligase degron recognition have contributed more broadly to the field of structural biology and small-molecule druggability by revealing the structural basis for PTM-dependent and small-molecule induced *de novo* formation of PPIs of functional relevance.

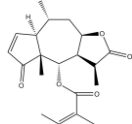
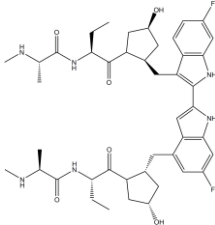
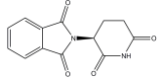
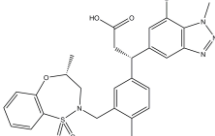
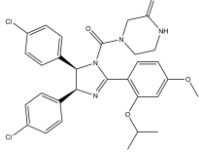
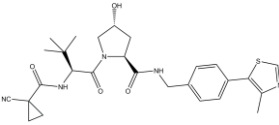
Small molecule approaches that enable conditional degradation of POIs, namely **small-molecule dependent inducible degrons and PROTACs**, represent complementary technologies and sophisticated chemical biology tools for post-translational protein inactivation. Targeted protein degradation is attracting increasing interest at both academic and pharmaceutical levels because of the potential to address therapeutic areas for which current methods are not suitable or are inadequate. Indeed, small molecules have been already used to induce rapid, selective depletion of key oncogenes or aberrant proteins in cells and *in vivo* disease models. We anticipate that this new modality of chemical intervention will impact increasingly relevant and yet un-drugged biological systems in the future.

Acknowledgements

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TABLES

Table 1. Small-molecule ligands of E3 ligases. E3 ligases, their recognized degron motifs, and examples of developed small-molecule ligands.

E3 ligase	Example substrate	Degron/structural motif/pattern	PDB of a protein–degron complex	Example of ligand (inhibitor/molecular glue)	PDB of the protein–ligand complex
β -TrCP	β -catenin, I κ B α	DpSGxxpS	1P22 [73]	6-O-angeloylplenolin 	n.a.
cIAP/XIAP	Caspase-3, SMAC	Substrate-targeting based on specific PPIs	1I3O [74]	Birinapant 	4KMP [75]
CRBN	MEIS2, IKZF1/3, CK1 α , GSPT1	Conserved structural loop with conserved Gly	5HXB [61], 5FQD [62]	Thalidomide 	4CI1 [57]
KEAP1	NRF2	[DNS]x[DES][TNS]GE	2FLU [76]	Compound 7 	5FNU [77]
MDM2	P53	FxxxWxx[VIL] forming an α -helix	1YCR [78]	Nutlin-3a 	4IPF [79]
VHL	HIF-1 α , HIF-2 α	LxxLAHyp	1LM8 [41], 1LQB [42]	VH298 	5LLI [48]

FIGURES

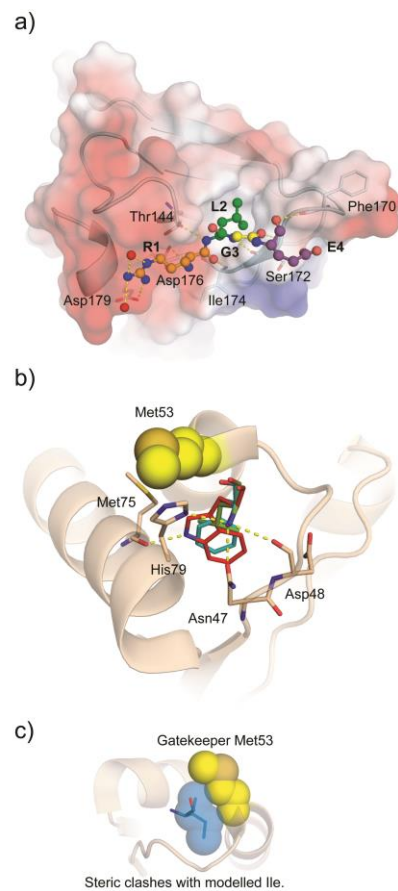


Figure 1. Structural basis of N-degron recognition. a) Crystal structure of ubiquitin ligase UBR1 from *S. cerevisiae* in complex with the type 1 N-degron substrate peptide RLGE (PDB code 3NIN [17]). The electrostatic potential surface of UBR1 is shown. b) Crystal structure of N-end rule adaptor protein ClpS from *C. crescentus* in complex with type 2 N-degron substrates Leu, Phe, and Trp (PDB codes 3G19, 3GQ1, and 3GW1, respectively [18]). Gatekeeper residue Met53 is highlighted. In a) and b), residues forming hydrogen bonds with the substrate are labelled. Note that in b), only Trp can interact by hydrogen bond with the backbone of Met75. c) Sphere representation of amino acid Ile modelled in PyMOL in the binding site of b) using PDB code 3G19.

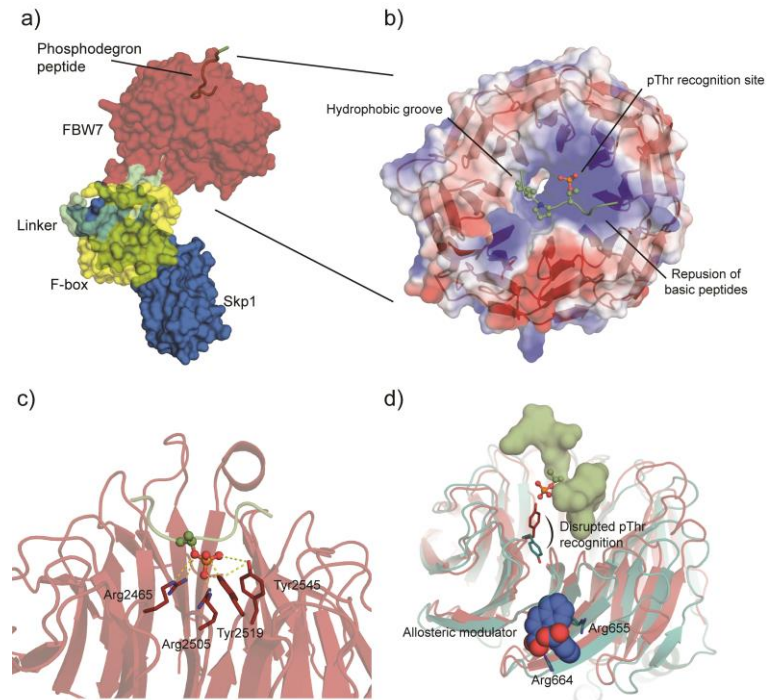


Figure 2. Structural basis of phosphodegrogen recognition. a) Crystal structure of human SCF^{FBW7} in complex with a phosphodegrogen peptide (PDB code 2OVR [30]). b) Apical view of the FBW7:peptide interaction (PDB code 2OVR). The electrostatic potential surface of FBW7 is shown. c) Closer view of the FBW7:phosphodegrogen hydrogen-bond interactions. Remarkably, only the phosphate group engages in polar interactions with the receptor. d) Superposition of the FBW7:phosphodegrogen complex and Cdc4 from *S. cerevisiae* in complex with an allosteric inhibitor (PDB code 3MKS [31]).

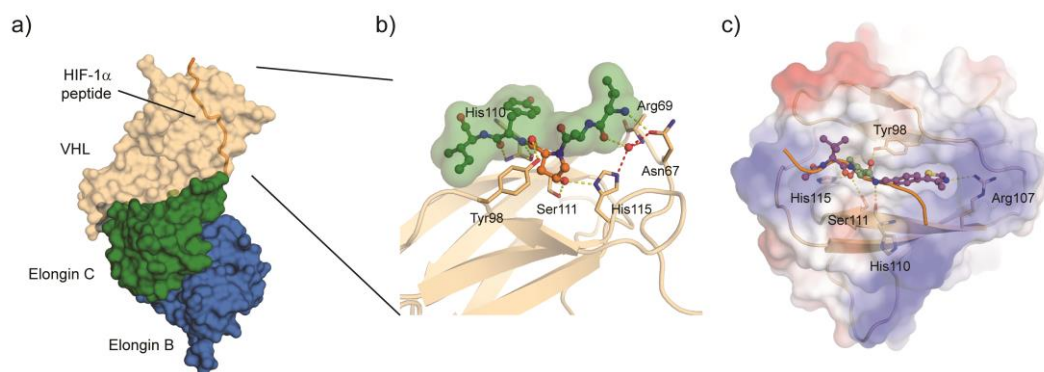


Figure 3. Structural basis of oxygen-dependent degron recognition. a) Crystal structure of human VHL:EloB:EloC:HIF-1 α (PDB code 1LM8 [41]). b) Closer view of the hydrogen-bond interactions in the VHL:HIF-1 α peptide complex (PDB code 1LM8). Notably, only the C⁴-*exo* pucker of Hyp can be satisfactorily accommodated in the pocket. In red dashed lines, hydrogen-bond interactions that stabilize a conserved water molecule. c) Apical view of the superposition of VHL in complex with HIF-1 α peptide and peptidomimetic VHL inhibitor VH032 (PDB codes 1LM8 and 4W9H, respectively [47]). The electrostatic potential surface of VHL is shown.

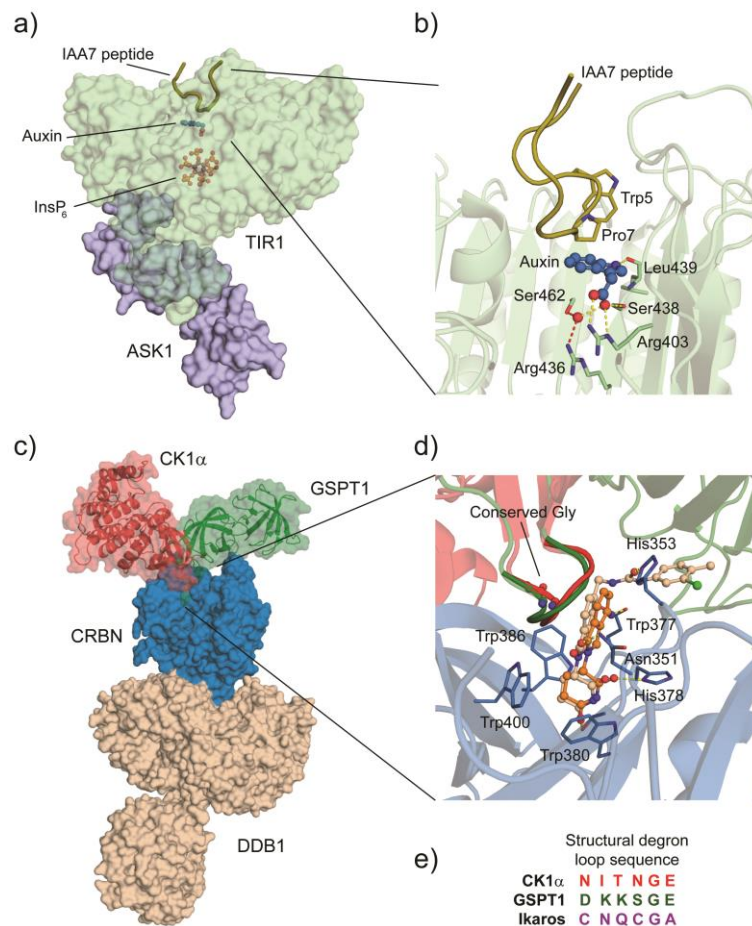
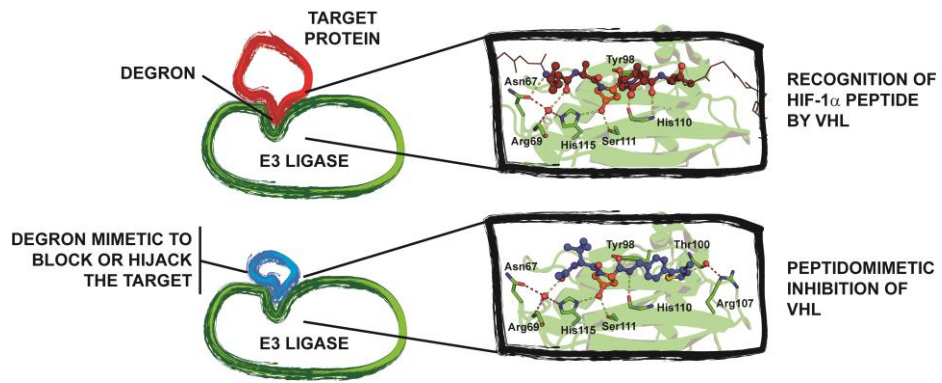


Figure 4. Structural basis of ligand-induced substrate recognition of CRLs. a) Crystal structure of SCF^{TIR1} from *A. thaliana* (PDB code 2P1M [50]). TIR1 binds a molecule of inositol-6-phosphate (InsP₆). b) Closer view of the hydrogen-bond interactions of TIR1 in complex with auxin and a IAA7 peptide (PDB code 2P1Q [50]). The auxin:IAA7 degnon peptide interaction is primarily driven by van der Waals packing. The hydrogen-bond network of a stabilized water molecule is shown in red dashed lines. c) Superposition of crystal structures of human CRL4^{CRBN}:CC-885:GSPT1 and CRL4^{CRBN}:lenalidomide:CK1 α complexes (PDB codes 5HXB and 5FQD, respectively [61, 62]). d) Closer view with highlighted residues on CRBN that form hydrogen bonds with the ligands (CC-885 in wheat and lenalidomide in orange). Note that Trp377 interacts only with lenalidomide, whereas CC-885 extends further reaching His353. Both compounds sit in a hydrophobic cavity of Trp residues. The topological conservation of the structural degnon loop of GSPT1 and CK1 α as recognized by CRBN and the conserved Gly residue are highlighted. The loop interacts with the small molecules primarily via van der Waals packing. e) Sequence alignment of the structural degnon loops in CK1 α , GSPT1, and Ikaros.

Figure for Graphical abstract



References

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