Ubiquitin-binding domains — from structures to functions

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Abstract | Ubiquitin-binding domains (UBDs) are modular elements that bind non-covalently to the protein modifier ubiquitin. Recent atomic-level resolution structures of ubiquitin–UBD complexes have revealed some of the mechanisms that underlie the versatile functions of ubiquitin *in vivo*. The preferences of UBDs for ubiquitin chains of specific length and linkage are central to these functions. These preferences originate from multimeric interactions, whereby UBDs synergistically bind multiple ubiquitin molecules, and from contacts with regions that link ubiquitin molecules into a polymer. The sequence context of UBDs and the conformational changes that follow their binding to ubiquitin also contribute to ubiquitin signalling. These new structure-based insights provide strategies for controlling cellular processes by targeting ubiquitin–UBD interfaces.

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Ubiquitin is a cellular signal that labels proteins in a highly controlled manner. Conjugation of ubiquitin to a target protein or to itself is regulated by the sequential activity of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligating (E3) enzymes, and it typically results in the addition of a ubiquitin moiety either to the ε-amino group of a Lys residue or to the extreme amino terminus of a polypeptide[1–3] (FIG. 1). Ubiquitin chains can grow as E2 and/or E3 enzymes catalyse the formation of an isopeptide bond between a carboxyl group of ubiquitin Gly76 and an ε-amino group of another ubiquitin's Lys. There are seven Lys residues in ubiquitin (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63), allowing for seven possible homotypic linkage types and multiple possible heterotypic chains[4]. As a consequence, cellular proteins are modified by various ubiquitin signals: monoubiquitin, multiple monoubiquitin marks or ubiquitin chains, which can be of diverse length and linkage[4].

Whereas the addition of a single ubiquitin to a target protein (monoubiquitylation) can alter protein activity and localization (by regulating endocytosis, lysosomal targeting, meiosis and chromatin remodelling), the formation of a diverse array of ubiquitin chains (polyubiquitylation) is implicated in events such as targeting to the 26S proteasome, immune signalling and DNA repair. The most recently described type of ubiquitin polymers are the linear (head-to-tail) ubiquitin chains, which are assembled by a specific ligase complex called the linear ubiquitin chain assembly complex (LUBAC)[5,6] and are crucial for nuclear factor-κB (NF-κB) signalling[6–8].

In these processes, ubiquitin acts as a signalling component that can trigger molecular events in cells. It does this by operating as a reversible and highly versatile regulatory signal for ubiquitin-binding domains (UBDs) in cellular proteins, new varieties of which are still being discovered. Many molecular details of signal transmission from ubiquitylated proteins (substrates that are modified following various cellular stimuli) to effector proteins (ubiquitin receptors containing one or more UBDs) have been elucidated in the past decade (FIG. 2).

In this Review, we discuss how different ubiquitin signals can be recognized by the distinct UBDs that are present in a multitude of cellular proteins (currently estimated to be more than 150). Different mechanisms have evolved to achieve *in vivo* specificity in the UBD–ubiquitin interactions, including increased avidity (the combined synergistic strength of bond affinities during multifaceted interactions), distinct affinities for ubiquitin chains of specific linkage, contributions by UBD-independent sequences and, finally, conformational changes following the UBD–ubiquitin interactions. These factors create environments in which the effective affinity and selectivity of ubiquitin–UBD interactions are functionally relevant in the context of a living cell. We also provide several recent examples in which structural and functional analyses were essential for uncovering the molecular basis of cellular processes, such as the regulation of protein stability, receptor trafficking in the endosome, DNA damage responses and inflammatory pathways. Moreover, we describe defects in ubiquitin–UBD interactions that are relevant for the development

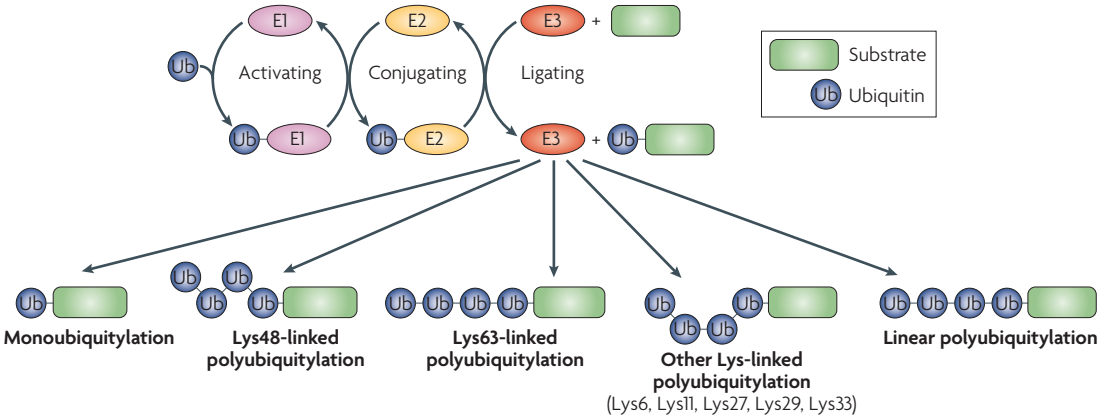


Figure 1 | **Enzymatic cascade that leads to substrate ubiquitylation.** The activity of three enzymes is required for ubiquitylation: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-ligating enzyme (E3), which recognizes the substrate. The completion of one ubiquitylation cycle results in a monoubiquitylated substrate. However, the cycle can be repeated to form polyubiquitylated substrates. Additional ubiquitin molecules can be ligated to a Lys residue (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 or Lys63) in a previously attached ubiquitin to form Lys-linked chains. Alternatively, ubiquitin molecules can be linked head to tail to form linear chains. Only homotypic ubiquitin chains are shown; however, some E2–E3 combinations can produce ubiquitin chains of mixed linkage.

26S proteasome
A large protein complex that carries out regulated degradation to control protein lifespan. Proteasome activity is essential for a large range of cellular events, including cell cycle progression, DNA repair, apoptosis and the removal of misfolded proteins.

Endosome
A small vesicle that is formed by endocytosis (invagination) of the plasma membrane, along with surface receptors, and is responsible for sorting internalized proteins and other biomolecules. Depending on the cargoes, endosomes are transported back to the cell surface for recycling, transported to the Golgi apparatus or matured into lysosomes.

β-Sheet
A secondary structural element of proteins, in which the peptide backbone is almost fully extended.

3_{10} Helix
A secondary structural element of proteins, in which a coiled conformation enables the formation of hydrogen bonds between backbone carbonyl and amide groups of amino acids that are three residues apart.

α-Helix
A secondary structural element of proteins, in which a coiled conformation enables the formation of hydrogen bonds between backbone carbonyl and amide groups of amino acids that are four residues apart.

Residual dipolar coupling
(RDC). Dipole–dipole coupling between spin ½ nuclei in samples that are partially aligned with an external magnetic field. RDC provides the orientation of bonds between neighbouring atoms relative to the rest of the molecule and can therefore be used in structure determination by NMR spectroscopy.

of diseases such as inflammation and cancer, and we elaborate on the design of new therapeutic approaches that can target ubiquitin–UBD interaction surfaces.

Ubiquitin: a diverse cellular signal

Ubiquitylation is among the most widely used protein modifications involved in regulating cellular signalling and homeostasis. At the molecular level, ubiquitin can be viewed as an intracellular signal that is inducibly and reversibly attached to a range of proteins and, as such, regulates a multitude of cellular functions. Ubiquitin has a diverse surface architecture and forms differently coupled chains, thus expanding its capacity to act as a versatile signalling messenger.

Monoubiquitin — one fold, many dynamic conformations. The canonical ubiquitin fold is formed by a 5-stranded β-sheet, a short 3_{10} helix and a 3.5-turn α-helix (FIG. 3a). The carboxy-terminal tail of ubiquitin is exposed, allowing its covalent linkage to target proteins. Ubiquitin is a small protein of 76 amino acids with a surface area of only 4,800 Å^2. Most UBDs interact with a solvent-exposed hydrophobic patch, which includes Leu8, Ile44 and Val70 and is located in ubiquitin's β-sheet (FIG. 3a). Although most UBDs tend to target the same surface of ubiquitin, the amino acids that surround the hydrophobic patch are chemically diverse. As a consequence, the more than two dozen UBDs characterized so far (TABLE 1) include a myriad of structural folds and unique binding modes.

X-ray and **nuclear magnetic resonance (NMR) structures** of ubiquitin bound to different UBDs indicate that ubiquitin adopts slightly different conformations depending on its binding partner. One study used NMR residual dipolar couplings (RDCs) to analyse the full dynamic behaviour of ubiquitin in solution on the nanosecond to microsecond timescale[9]. This showed that free ubiquitin exhibits the structural heterogeneity of its UBD-bound states, as each state corresponded to a member of the ensemble of structures sampled by free ubiquitin over time (FIG. 3b). This structural heterogeneity contributes strongly to the adaptive interface of ubiquitin when binding to different UBDs.

Ubiquitin chains — diverse cellular signals. An attractive model that describes how the outcome of ubiquitylation is determined in cells proposed that the fate of ubiquitylated substrates depends on the type of ubiquitin linkage and the length of the ubiquitin chain[10]. This early model applies best to ubiquitin chains that are homogeneous, namely those that are assembled through a single linkage type. Structural studies have revealed significant differences between ubiquitin chains of Lys48 (FIG. 3c) and Lys63 (FIG. 3d) and of linear linkages (FIG. 3e). Lys63-linked and linear diubiquitins adopt similar extended conformations, with no contact between the two ubiquitin moieties[11]. By contrast, the ubiquitin moieties of Lys48-linked tetraubiquitin pack against each other[12,13] and alternate in solution between a closed, packed structure and an extended, open one[14].

Importantly, structures of ubiquitin chains in complex with UBDs have revealed that Lys48-linked, Lys63-linked and linear linkages of ubiquitin molecules adopt a range of inter-moiety arrangements that depend on the UBD (see Supplementary information S1 (figure)). For example, the open conformation of Lys63-linked diubiquitin is stretched when in complex with the endosome-associated ubiquitin isopeptidase AMSH (associated molecule with the Src homology 3 domain of signal-transducing adapter molecule) to allow efficient cleavage of the isopeptide bond[15]. By contrast, Lys63-linked diubiquitin in complex with an antibody adopts a more compact conformation in which the two ubiquitin moieties are packed together, with substantial surface contacts between them[16]. These structural studies have led to ubiquitin chains being viewed as flexible modules that can be shaped by their cellular context.

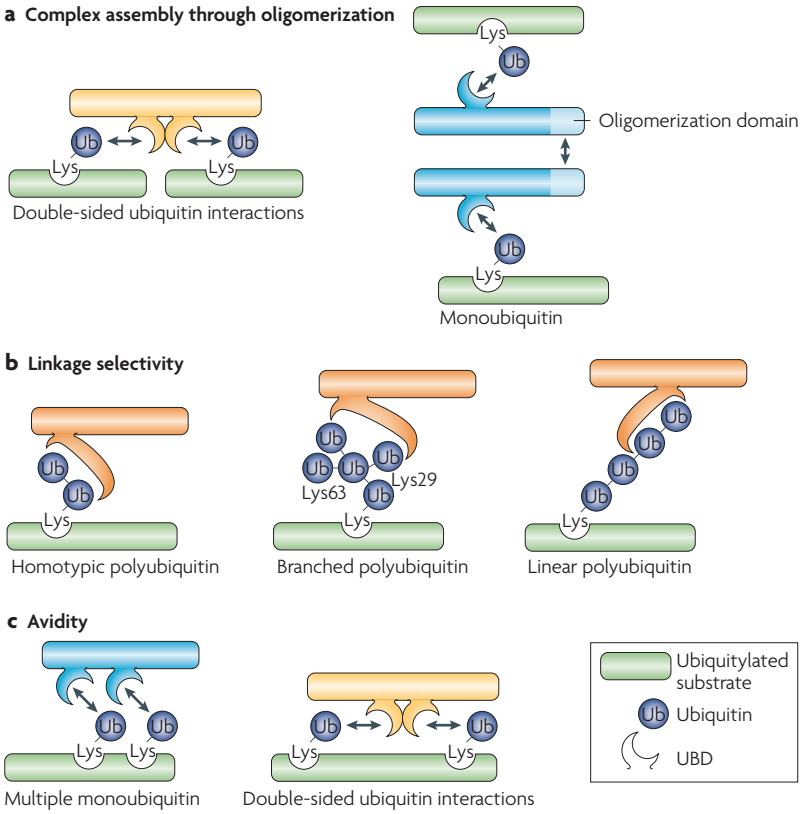


Figure 2 | **The ubiquitin–UBD network.** Ubiquitin can be covalently attached to target proteins as a single moiety (monoubiquitin), as multiple single moieties (multiple monoubiquitin), as chains coupled through the same Lys residue in ubiquitin (homotypic polyubiquitin), as mixed chains linked through different Lys residues in ubiquitin (branched polyubiquitin) or as head-to-tail bound ubiquitin moieties (linear polyubiquitin). Specialized sets of ubiquitin-binding domains (UBDs) can read these post-translational modifications and mediate different outputs depending on the protein in which they are embedded. **a** | Two UBDs in the same protein can bridge two ubiquitylated substrates. Alternatively, two proteins carrying oligomerization domains and UBDs can indirectly bridge the same ubiquitylated substrate. In both cases this results in the formation of protein complexes, which might help to amplify a signal or activate a downstream process. **b** | Specialized UBDs have also been discovered that can selectively discriminate between different types of ubiquitin chains. **c** | The presence of two or more UBDs in a protein or the attachment of multiple ubiquitin moieties on the same substrate can increase the avidity of and promote ubiquitin–UBD interactions, despite the low affinities of the individual interactions. This phenomenon might be important to filter noise coming from nonspecific transient ubiquitin–UBD interactions and to amplify only the output of proper ubiquitin–UBD pairs. UBDs that bind to one ubiquitin moiety are shown in blue, those that interact specifically with the regions linking ubiquitin moieties are in orange, and double-sided UBDs are in yellow. Arrows indicate protein–protein interactions.

Zinc finger
A small structural motif in proteins that is stabilized by interactions between amino acid side-chain atoms and a coordinated zinc atom.

Ubiquitin conjugating (UBC) domain
The α/β structural fold of E2 conjugating enzymes that is characterized by a conserved active-site Cys residue, which forms a thiolester bond with ubiquitin.

UBDs — multiple folds for ubiquitin

UBDs are diverse modules in a protein that can bind, and often distinguish, different types of ubiquitin modifications. The number of identified UBDs is constantly growing, with more than twenty different families identified to date (TABLE 1). UBDs diverge both in structure and in the type of ubiquitin recognition that they use. Most commonly, they fold into α-helical structures (FIG. 4a), zinc fingers (ZnFs) (FIG. 4b), the ubiquitin-conjugating (UBC) domains present in E2 enzymes (FIG. 4c), or plekstrin homology (PH) folds (FIG. 4d,e). Several comprehensive reviews have described the various classes of UBDs and their biological properties[17–21]. In the following subsections, we discuss the principles by which distinct classes of UBDs interact with ubiquitin modifications.

UBDs that bind monoubiquitin. Most UBDs use α-helical structures to bind a hydrophobic patch in the β-sheet of ubiquitin. The ubiquitin-interacting motif (UIM), inverted UIM (IUIM; also known as motif interacting with ubiquitin (MIU)) and ubiquitin-binding zinc finger (UBZ) bind this region of ubiquitin with a single α-helix[22–27] oriented either parallel or antiparallel to the central β-strand (FIG. 4b and see Supplementary information S2 (figure)). Other ubiquitin-binding elements, including the ubiquitin-associated (UBA) domain (FIG. 4a) and the coupling of ubiquitin conjugation to endoplasmic reticulum degradation domain (CUE domain)[28–31], bind ubiquitin through two discontinuous α-helices. It is currently unclear why so many variations of helical structures have evolved to interact with ubiquitin and regulate its downstream signalling. Several examples of the involvement of α-helices in specific ubiquitin signalling to regulate proteasomal functions, DNA damage response or receptor endocytosis are discussed below.

The β-sheets that are present in different structural domains of deubiquitinases (DUBs; also known as deubiquitylating or deubiquitinating enzymes) can also engage in the recognition of monoubiquitin. For example, the β-sheet of the ubiquitin-conjugating enzyme UBCH5C (also known as UBE2D3) interacts with ubiquitin's hydrophobic Ile44-containing surface[32] (FIG. 4c). UBCH5C functions with the E3 ligase breast cancer type 1 susceptibility protein (BRCA1), a tumour suppressor protein with breast cancer-associated mutations. The non-covalent interaction between UBCH5C and ubiquitin activates the self-assembly of ubiquitin chains on UBCH5C and, in turn, activates processive ubiquitylation by BRCA1 (REF. 32), which is crucial for coordinating DNA damage regulatory complexes (see below). Another example is the GRAM-like ubiquitin-binding in EAP45 (GLUE) domain that is present in the ELL-associated protein of 45 kDa (EAP45; also known as VPS36)) subunit of ESCRT (endosomal sorting complexes required for transport). This GLUE domain folds into a split PH domain that also uses residues of β-strands S5 and S6 to bind the hydrophobic patch of ubiquitin, although, in this case, additional residues from a loop and an α-helix are also used[33,34] (FIG. 4d). The PH receptor for ubiquitin (PRU) domain present in the proteasomal receptor regulatory particle, non-ATPase-like 13 (Rpn13) binds to the β-strand surface of ubiquitin with a PH domain; however, it does so in an entirely different manner to the GLUE domain[35]. Three loops in the PRU domain of Rpn13 form a binding interface that contacts a larger surface than the split PH domain of EAP45, and forms hydrogen bonds with the His68 in ubiquitin (FIG. 4e). These properties of the PRU domain contribute to its higher affinity for monoubiquitin[36]. Hence, even when the same structural domain is used to bind the Ile44-containing surface of ubiquitin, recognition can occur through different UBD surfaces and

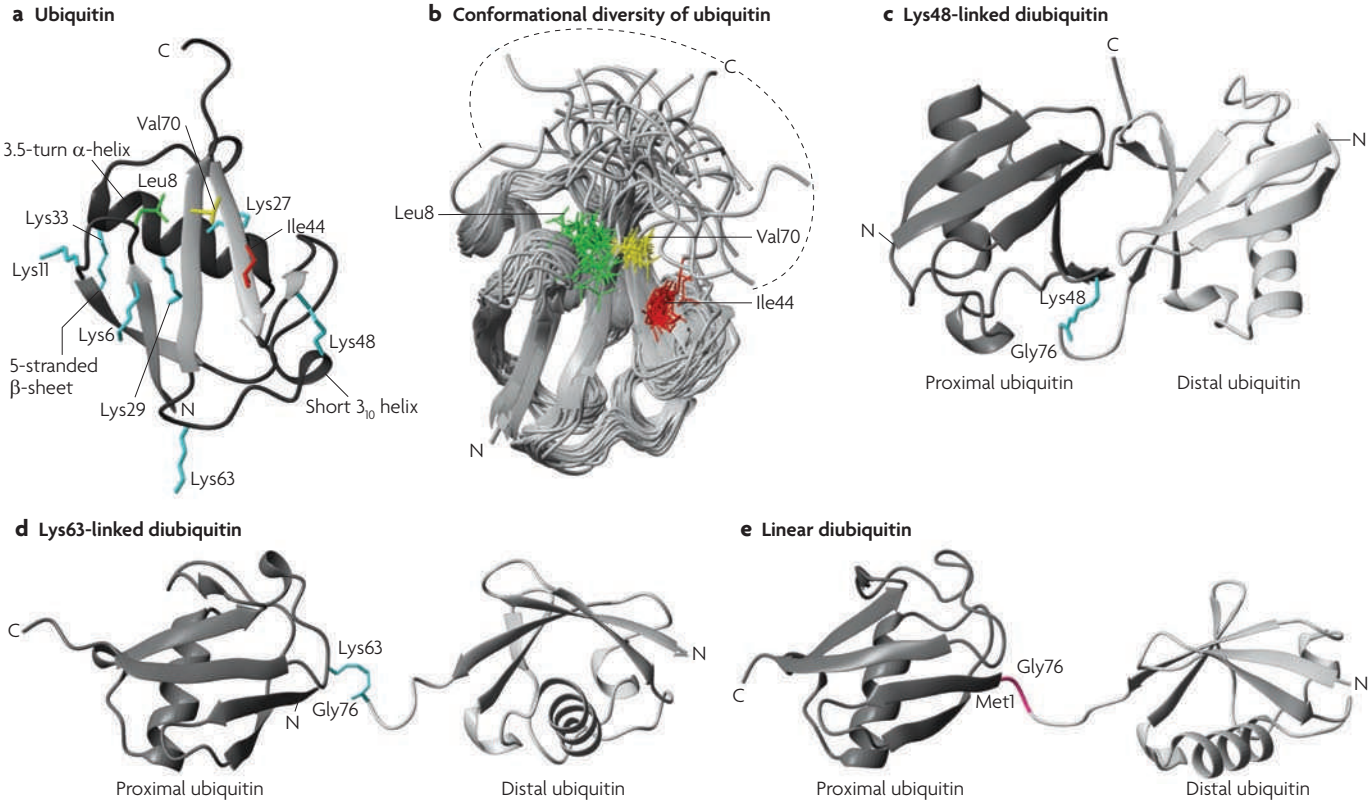


Figure 3 | **Structural diversity contributes to the multiplicity of ubiquitin signalling. a** | A ribbon representation of monoubiquitin (Protein Data Bank (PDB) identifier 1D3Z)[107]. Ubiquitin contains a 5-stranded β-sheet, a 3.5-turn α-helix and a short 3_{10} helix. Seven solvent-exposed Lys residues (blue) are available to assemble ubiquitin chains, and the hydrophobic residues Leu8 (green), Ile44 (red) and Val70 (yellow) serve as a platform for many ubiquitin-binding domain (UBD) interactions. **b** | Free ubiquitin in solution is a dynamic molecule with conformational diversity. Distinct conformations are selected by individual UBDs and several are shown here to highlight the dynamic range of motions that ubiquitin displays in solution[9]. **c** | Ribbon representation of Lys48-linked diubiquitin (PDB identifier 1AAR)[12]. Lys48-linked chains form compact structures as a result of inter-moiety interactions. The isopeptide bond linkage is shown in cyan. **d** | Ribbon representation of Lys63-linked diubiquitin (PDB identifier 2JF5). The isopeptide bond linkage is shown in cyan. **e** | Ribbon representation of linear diubiquitin, forming a peptide bond (magenta) between Met1 and Gly76 (PDB identifier 2W9N)[11]. Lys63-linked and linear ubiquitin chains have more extended conformations than Lys48-linked chains. Linkage of ubiquitin molecules into a polymer enhances the structural diversity for robust signalling. In each case, the linker and its neighbouring region are chemically diverse.

Plekstrin homology (PH) fold
A structural fold in proteins that is characterized by a distinct pattern of β-strands and an α-helix. This protein family tends to have a core of bulky hydrophobic amino acids.

Ubiquitin-binding zinc finger
(UBZ). A subclass of ubiquitin-binding zinc finger domain that can bind to ubiquitin and control DNA damage responses.

Ubiquitin-associated (UBA) domain
A small structural domain in proteins that is characterized by a three-helix bundle and is typically associated with the ubiquitin pathway.

structural elements. This finding highlights the difficulty of identifying UBDs and of predicting ubiquitin-binding surfaces without experimental data.

The common use of the Ile44-containing surface by UBDs causes the binding of UBDs to monoubiquitin to be mutually exclusive, suggesting that in most cases a ubiquitin moiety interacts with only one UBD. It is possible that this mutual exclusivity is an important component of effective ubiquitin signalling, as it can prevent each ubiquitin from stimulating multiple processes, which could lead to discordance within the cell. An exception to this rule is seen with the variations in ZnF domains. These domains recognize monoubiquitin by binding to three different regions on its surface (FIG. 4b). The nuclear protein localization 4 ZnF (NZF) domain[37], which serves as a ubiquitin-binding adaptor protein in the endoplasmic reticulum-associated degradation (ERAD) pathway, and the ubiquitin-binding ZnF (UBZ) domain, which is present in translesion synthesis polymerases[38], bind to the Ile44-containing hydrophobic surface of the ubiquitin β-sheet. By contrast, the A20-type ZnF domain of RAB5 guanine nucleotide exchange factor (RABEX5; also known as RABGEF1) recognizes a polar surface of ubiquitin that is centred on Asp58 (REF. 24). This empowers RABEX5 to act with ligase capacity on substrates in *trans*. A different binding surface is contacted by the DUB isopeptidase T (IsoT), which has a ZnF domain that binds to the C-terminal residues of ubiquitin[39]. The unique ubiquitin-binding mode of the ZnF domain of IsoT ensures that it disassembles only unanchored chains[40], and suggests that it could act in conjunction with other UBDs that bind to ubiquitin's Ile44-containing surface. The impressive diversity of DUB structure and ubiquitin recognition strategies was recently described in an excellent review article[41].

There is no evidence that the binding affinity for ubiquitin can be predicted on the basis of the UBD structural fold. By contrast, a larger range of affinities is

Table 1 | **The functional and structural diversity of ubiquitin-binding domains**

Ubiquitin-binding domain	Representative protein*	Function	References
α-Helix			
UIM	S5a (human) and Rpn10 (yeast), Vps27, STAM, epsins and RAP80 (UIMC1)	Proteasome degradation, endocytosis, MVB biogenesis and DNA repair	26,27,77,110
IUIM (also known as MIU)	RABEX5	Endocytosis	24,25
DUIM	HRS	MVB biogenesis	23
UBM	Polymerase iota and reversionless 1	DNA damage tolerance	38
UBAN	NEMO, ABIN1–ABIN3 and optineurin	Nuclear factor-κB signalling	8,11,50,51
UBA	Rad23 (yeast) and R23A (human), Dsk2 and NBR1	Proteasome targeting, kinase regulation and autophagy	30,42,111, 112
GAT	GGA3 and TOM1	MVB biogenesis	58,60
CUE	Vps9, TAB2 and TAB3	Endocytosis and kinase regulation	29,113
VHS	STAM and GGA3	MVB biogenesis	114
Zinc finger (ZnF)			
UBZ	Polymerase-h; polymerase-k and Tax1BP1	DNA damage tolerance and nuclear factor-κB signalling	38,115
NZF	NPL4, Vps36, TAB2 (MAP3K7IP2) and TAB3 (MAP3K7IP3)	ERAD, MVB biogenesis and kinase regulation	37,116,117
ZnF A20	RABEX5 (RABGEF1) and A20 (TNFAIP3)	Endocytosis and kinase regulation	24,25
Znf UBP (also known as PAZ)	Isopeptidase T (USP5) and HDAC6	Proteasome function, aggresome function and autophagy	39,118
Plekstrin homology (PH) domain			
PRU	RPN13	Proteasome function	35,36
GLUE	EAP45 (VPS36)	MVB biogenesis	35,36
Ubiquitin-conjugating (Ubc)-like domain			
UEV	UEV1 (UBE2V1) and MMS2	DNA repair, MVB biogenesis and kinase regulation	119,120
UBC	UBCH5C (UBE2D3)	Ubiquitin transfer	32
Others			
SH3	Sla1 and CIN85 (SH3KBP1)	Endocytosis	121
PFU	Ufd3 (Doa1)	ERAD	122
Jab1/MPN	Prp8	RNA splicing	123

*Alternative protein names are provided in brackets. ABIN, A20 binding inhibitor of nuclear factor-κB protein; CUE, coupling of ubiquitin conjugation to endoplasmic reticulum degradation; DUIM, double-sided UIM ; Dsk2, dominant suppressor of KAR1; EAP45, ELL-associated protein of 45 kDa; ERAD, endoplasmic reticulum-associated degradation; GAT, GGA and TOM; GGA, Golgi-localized, γ-ear-containing, ARF-binding; GLUE, GRAM-like ubiquitin-binding in EAP45; HDAC6, histone deacetylase 6; HRS, hepatocyte growth factor-regulated tyrosine kinase substrate; IUIM, inverted UIM; MVB, multivesicular body; NBR1, next to BRCA1 gene 1; NEMO, nuclear factor-κB essential modulator; NPL4, nuclear protein localization 4; NZF, nuclear protein localization 4 ZnF; PFU, PLAA family ubiquitin binding; PRU, plekstrin homology receptor for ubiquitin; RABEX5, RAB5 guanine nucleotide exchange factors; RAP80, receptor associated protein 80; RPN13, regulatory particle, non-ATPase-like 13; SH3, Src homology 3; STAM, signal-transducing adapter molecule; TAB, TAK1 binding protein; TOM, target of Myb; UBA, ubiquitin-associated; UBAN, ubiquitin binding in ABIN and NEMO; UBC, ubiquitin-conjugating; UBM, ubiquitin-binding motif; UBP, ubiquitin-binding protein; UBZ, ubiquitin-binding ZnF; UEV, ubiquitin-conjugating enzyme E2 variant; UIM, ubiquitin-interacting motif; VHS, VPS27, HRS and STAM; VPS, vacuolar protein sorting-associated protein.

found in the UBA domain family than is found between UBA domains and UBZ or CUE domains. There is also no clustering of particular UBD folds into specific effector protein types or into selective cellular functions. Rather, the same class of UBD, for example UBA domains, is found in various proteins, with numerous additional domains implicated in a range of cellular functions that include endocytosis, proteasome signalling and apoptosis.

Lys linkage-specific UBDs. Linkage-specific ubiquitin recognition contributes to the diverse set of functional outcomes associated with ubiquitylation. The pioneering work of Cecile Pickart and colleagues revealed diversity in ubiquitin chain recognition preferences for 30 different UBA domains. Although some UBA domains showed little discretion between ubiquitin chains of different linkage, others preferred Lys48-linked ubiquitin chains[42]. One of the radiation sensitivity abnormal 23 (Rad23) human

CUE domain
(Coupling of ubiquitin conjugation to endoplasmic reticulum degradation domain). A protein structural domain that is similar to the UBA domain. CUE domains form a three-helix bundle.

ERAD
(Endoplasmic reticulum-associated degradation). A pathway by which misfolded proteins are transported from the endoplasmic reticulum to the 26S proteasome in the cytosol.

Translesion synthesis polymerase
A member of a group of DNA polymerases that carry out translesion synthesis past DNA lesions. These polymerases, mostly belonging to the Y-family, have an open configuration and can accommodate different damaged bases in their active sites.

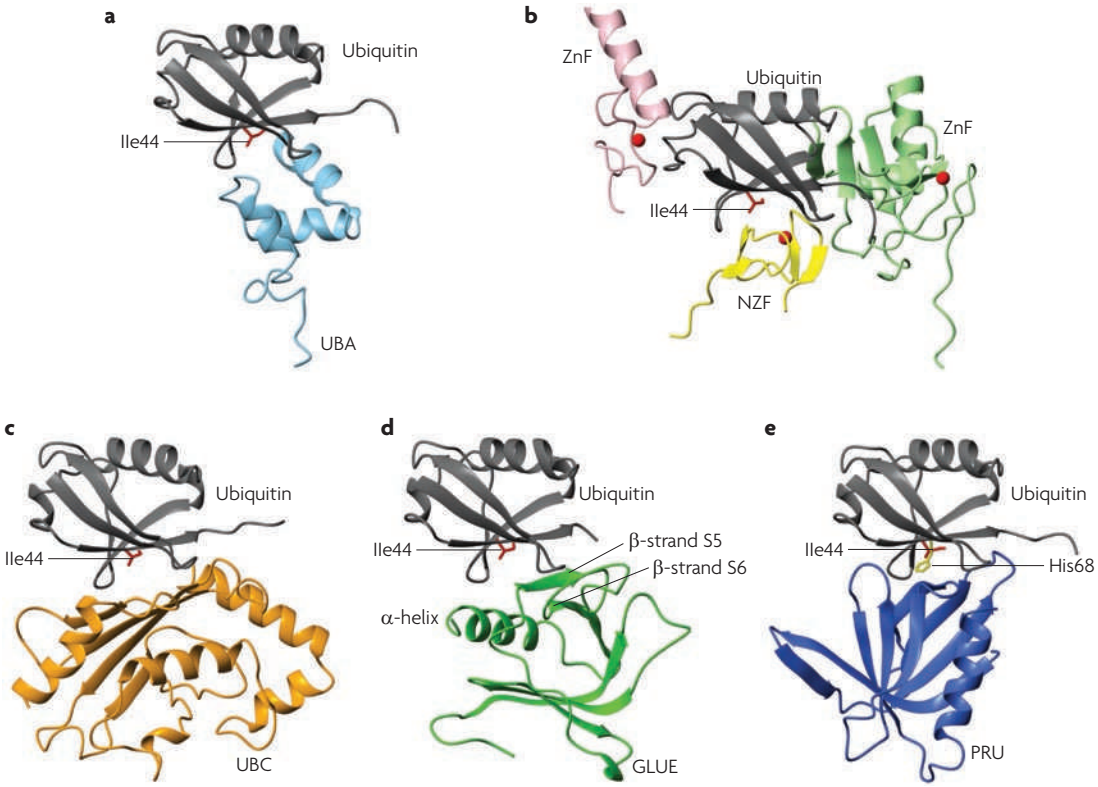


Figure 4 | **Ubiquitin is recognized by structurally diverse domains.** The structures of several ubiquitin–ubiquitin-binding domain (UBD) complexes. Ubiquitin (grey) is shown in the same orientation to highlight the common use of its β-strand surface by diverse UBDs. **a** | Ribbon representation of ubiquitin in complex with the ubiquitin-associated (UBA) domain of protein linking IAP with cytoskeleton 1 (PLIC1; light blue) (Protein Data Bank (PDB) indentifier 2JY6)[108]. **b** | Ribbon representation of ubiquitin in complex with the A20-type zinc finger (ZnF) domain (yellow) of RAB5 guanine nucleotide exchange factor (RABEX5; also known as RABGEF1; light green) (PDB indentifier 2FIF)[24], the nuclear protein localization 4 (NPL4) ZnF (NZF) domain (PDB indentifier 1Q5W)[37] and the ZnF domain of the deubiquitinase (DUB; also known as deubiquitylating or deubiquitinating enzyme) isopeptidase T ubiquitin-binding protein (UBP; pink) (PDB indentifier 2G45)[39]. The NZF domain binds to the Ile44-centred surface, the A20 ZnF domain of RABEX5 binds to an Asp58-centred surface and the ZnF domain of isopeptidase T UBP binds to the carboxyl terminus. Zinc atoms are shown as red spheres. **c** | Ribbon representation of ubiquitin in complex with the E2 ubiquitin-conjugating enzyme UBCH5C (also known as UBE2D3; orange) (PDB indentifier 2FUH)[32]. **d** | Ribbon representation of ubiquitin in complex with the GRAM-like ubiquitin-binding in EAP45 (GLUE) domain of ELL-associated protein of 45 kDa (EAP45; also known as VPS36; dark green) (PDB identifier 2DX5)[33]. **e** | Ribbon representation of ubiquitin in complex with the plekstrin homology for ubiquitin (PRU) domain of regulatory particle, non-ATPase-like 13 (RPN13; dark blue) (residues 1–150; PDB identifier 2Z59)[35]. The side chain atoms of His68 are shown in yellow. The three-helix bundle structure of the UBA, E2 ubiquitin-conjugating and plekstrin homology domains binds to the Ile44-centred hydrophobic patch in ubiquitin, but does so in diverse manners and cannot simultaneously act on a common monoubiquitin or ubiquitin moiety within a chain. By contrast, ZnF domains are more diverse in the manner by which they bind to ubiquitin.

homologues, RAD23A, which as discussed below is a ubiquitin receptor that is associated with substrate targeting to the 26S proteasome, has a C-terminal UBA domain that binds with 3.6-fold higher affinity to Lys48-linked chains than to Lys63-linked ones[42]. This C-terminal UBA domain, like many other UBDs, binds to monoubiquitin with 70-fold lower affinity than it binds to Lys48-linked ubiquitin chains[42]. The structure of the RAD23A C-terminal UBA domain–Lys48-linked diubiquitin complex revealed that the UBA sandwiches between the two ubiquitin moieties to form unique contacts (including some interactions with the diubiquitin linker region) and a significantly larger binding surface than it could form with a single ubiquitin. This structure explains its preference for the Lys48 linkage[43] (FIG. 5a).

Several UBDs can selectively bind to Lys63-linked ubiquitin chains. For example, NZF domains of TAK1-binding protein 2 (TAB2; also known as MAP3K7IP2) or TRAF-binding protein domain (TRABID; also known as ZRANB1) preferentially bind to Lys63- over Lys48-linked ubiquitin chains[11]. The specificity determinants that promote the interaction of NZF domains with ubiquitin chains of a certain linkage type are currently unknown.

In contrast with other proteins that contain UBDs, DUBs must act at regions that link ubiquitin moieties in a chain in order to access the cleavage site between the ubiquitin moieties. This family of proteins in particular exhibits a high degree of specificity for certain linkage types. For example, the DUBs IsoT and ubiquitin

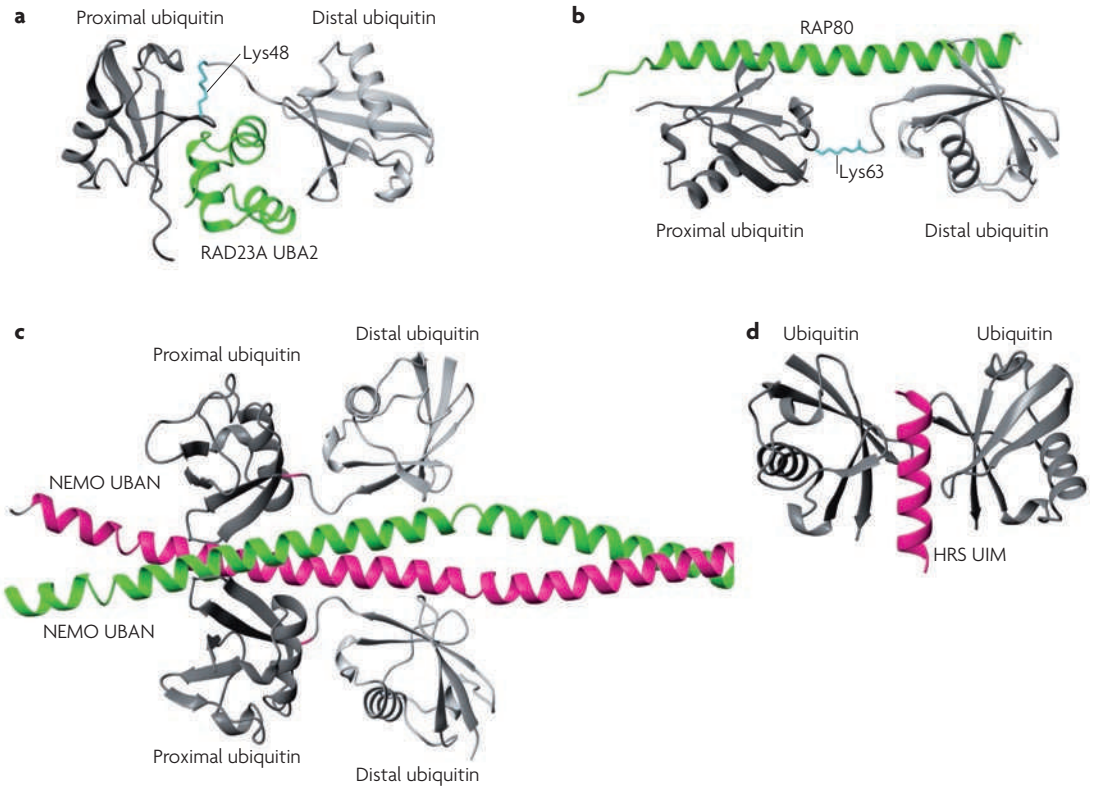


Figure 5 | **Multivalent interactions between ubiquitin and UBDs define chain specificity and increase affinity.** **a** | The ubiquitin-associated 2 (UBA2) domain (green) of a radiation sensitivity abnormal 23 human homologue, RAD23A, is sandwiched between the two ubiquitin moieties of Lys48-linked diubiquitin (grey) (Protein Data Bank (PDB) indentifier 1ZO6)[43]. This structure provides an explanation for the preference of this domain for Lys48-linked chains: it contacts the ubiquitin linker region to expand its binding surface beyond that possible for monoubiquitin. **b** | Structure of Lys63-linked diubiquitin bound to receptor associated protein 80 (RAP80), illustrating why RAP80 binds to Lys63-linked, but not Lys48-linked, chains (coordinates provided by S. Fukai, University of Tokyo, Japan)[109]. Its contiguous helix binds simultaneously to two ubiquitin moieties, thereby increasing its affinity beyond that possible with monoubiquitin and making the spacing between the two ubiquitin moieties greater than would be achievable with the Lys48 linkage. **c** | The UBAN (ubiquitin binding in ABINs (A20-binding inhibitor of nuclear factor-κB proteins) and NEMO (nuclear factor-κB essential modulator)) domain in NEMO forms a coiled coil, which binds two linear diubiquitins[7,8]. Extensive contacts are formed with both ubiquitin moieties, thus conveying specificity for linear ubiquitin chains. **d** | The structure of the single-helix, double-sided ubiquitin-interacting motif (UIM) of hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) bound to two ubiquitins reveals how it can target two ubiquitin molecules with equal affinity (PDB indentifier 2D3G)[23]. The ubiquitin molecules interact on opposite sides of the UIM and with a similar binding mode.

C-terminal hydrolase 2 (USP2) readily cleave Lys48- and Lys63-linked chains, but they have less activity towards linear chains[11]. By contrast, the DUB cylindromatosis tumour suppressor (CYLD), which is a negative regulator of NF-κB signalling[44], cleaves linear and Lys63-linked chains but not Lys48-linked ones[11]. Other examples of DUBs with explicit specificity are some of those containing ovarian tumour (OTU) domains. The A20 OTU domain hydrolyses only Lys48-linked chains, whereas the TRABID OTU preferentially binds and cleaves Lys63-linked chains[11].

Linker regions in tandem repeats of UBDs can also define linkage specificity. For example, receptor associated protein 80 (RAP80; also known as UIMC1) targets BRCA1 to DNA damage-induced foci[45–47] through its two UIMs, which bind Lys63-linked ubiquitin chains but not Lys48-linked ones[48]. The sequence between the two UIMs of RAP80 promotes an appropriate protein conformation such that the UIMs are positioned for efficient avid binding across a single Lys63 linkage, thus defining selectivity[48] (FIG. 5b). By contrast, ataxin 3, a DUB linked to the development of spinocerebellar ataxia type 3, contains two UIMs with a two-residue linker sequence that determines Lys48-specific binding[48,49]. The specificity of tandem UIMs can be changed between Lys63 and Lys48 linkages by swapping the linker sequences of RAP80 and ataxin 3 (REF. 46).

Thus, tandem UIMs can be spatially arranged in the context of full-size proteins, such that simultaneous, high-affinity interactions are favoured by one ubiquitin chain linkage but are unfavoured or impossible with other linkages. Hence, the coordinated action of multiple UBDs can be used to sort substrates towards specific functional pathways according to their ubiquitin

chain linkage type. These findings have provided a molecular basis for the observation that Lys63-linked chains have a role in DNA repair rather than proteasome targeting.

UBDs specific for linear ubiquitin chains. Linear ubiquitin chains, in which ubiquitin monomers are conjugated through Gly to Met linkages (FIG. 3e), have been implicated in the activation of the NF-κB signalling pathway[7,8]. Several proteins that regulate this pathway, including NF-κB essential modulator (NEMO), A20-binding inhibitor of NF-κB proteins (ABINs) and optineurin, contain the ubiquitin binding in ABIN and NEMO domain (UBAN domain), which specifically binds to linear ubiquitin chains[8,11,50,51]. The *in vitro* binding of the UBAN domain of NEMO to monoubiquitin is undetectable and its binding to Lys63-linked diubiquitin is 100-fold weaker than to linear chains[8,50]. Specific mutations of NEMO that block interactions with linear ubiquitin chains impair the activation of IκB kinase (IKK) and NF-κB in response to tumour necrosis factor-α (TNFα) stimulation[8].

The UBAN domain of NEMO forms a heterotetrameric complex with two linear diubiquitin molecules on either side of the UBAN coiled-coil dimer[8] (FIG. 5c). Specificity for linear ubiquitin chains is provided by a continuous surface along the coiled coil that interacts with the canonical Ile44-containing surface, the C-terminal tail of the distal ubiquitin and a unique interaction surface of the proximal ubiquitin[8]. Two loops, one from the distal ubiquitin molecule (from Pro37 to Gln40) and the other from the proximal ubiquitin (from Glu92 to Glu94), interact with each other and with the C-terminal tail of the distal ubiquitin (from Arg72 to Gly76). These residues form a structural core, which holds the two moieties in a semi-fixed orientation and provides the basis for specificity towards linear ubiquitin chains.

The specificity of UBDs for ubiquitin linkages can explain the *in vivo* activation of the NF-κB pathway. The NZF domain of TAB2 binds preferentially to Lys63 chains and is important for the recruitment and activation of TAK1, a Ser/Thr kinase that is required for activation of the NF-κB and mitogen-activated protein kinase (MAPK) pathways upon stimulation by TNF[52]. By contrast, linear ubiquitin chains affect the IKK complex through direct binding to the UBAN domain in neighbouring NEMO, which in turn can activate the NF-κB pathway[7,8].

UBDs specific for linear ubiquitin chains are probably involved in the regulation of additional cellular processes. For example, the UBAN-containing protein ABIN1 (also known as TNIP1) binds preferentially to linear chains and was suggested to act as a negative regulator of the NF-κB pathway[50]. However, embryonic fibroblasts from *Abin1*$^{-/-}$ mice do not have any defect in TNFα-induced NF-κB activation. *Abin1*$^{-/-}$ mice die during embryonic development as a result of massive apoptosis in the liver. It has been suggested that ABIN1 functions as a ubiquitin-dependent, anti-apoptotic sensor in mice[53].

UBAN domain
(Ubiquitin binding in ABIN and NEMO domain). An α-helical domain that is present in NEMO, ABINs and optineurin. It binds specifically to linear ubiquitin chains.

Coiled coil
A structural motif in which α-helices coil around each other to enable favourable interactions between amino acid side-chain atoms.

Multivesicular body
(MVB). A specialized endosome that is formed from early and sorting endosomes through invagination of the membrane enriched with surface receptors, resulting in many internalized vesicles. MVBs mature into late endosomes and lysosomes for degradation of internalized surface receptors.

Multivalent ubiquitin–UBD interactions

More evidence is emerging that multiplication of UBDs in an effector protein or associated complexes can provide a multivalent binding surface for long or complex ubiquitin chains. Recent evidence indicates the functional importance of such arrangements in the regulation of endosomal sorting and DNA repair.

ESCRT-ing ubiquitylated cargoes. Endocytic sorting of ubiquitylated surface receptors, such as epidermal growth factor receptor (EGFR), for lysosomal degradation in multivesicular bodies (MVBs) is carried out by a series of ubiquitin receptors that are incorporated into larger complexes named ESCRTs[17,54–56]. The machinery includes four complexes (ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III) that are required for the sequential sorting of cargoes. The structural details of isolated components and their complexes have been revealed for each of the ESCRT assemblies in recent years[20,57].

The cores of these complexes are structurally conserved, mostly with α-helical domains. The regions responsible for ubiquitin recognition, UIM in ESCRT-0, ubiquitin-conjugating enzyme E2 variant (UEV) in ESCRT-I and NZF and PH in ESCRT-II, are found at the peripheries of the ESCRT complexes to enable efficient recruitment of ubiquitylated cargoes. As discussed below, there are substantial variations in the mode of ubiquitin recognition by these domains from yeast through to mammalian systems. Recognition of multiple ubiquitin moieties attached to a trafficking cargo must be robust to control sorting of often bulky cargoes, but it cannot be so strong as to prevent efficient and seamless trafficking[20,54]. The appropriate level of ubiquitin interaction with ESCRT machineries is accomplished by the multivalency of the ubiquitin-recognizing subunits and by local concentration of ubiquitin-linked cargoes.

A remarkable example of multivalent interactions is the use of double-sided ubiquitin-binding modes in which α-helical (single, double and triple) structures form two ubiquitin-binding surfaces. Hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) is a subunit of ESCRT-0, and its UIM, which forms a short α-helix, binds two ubiquitin molecules with equal affinity. The crystal structure of the UIM in complex with two ubiquitin molecules shows that both ubiquitin moieties use the canonical Ile44-containing patch but interact with the UIM on opposing sides with pseudo two-fold symmetry[23] (FIG. 5d). This binding mode is accomplished by a shift of two residues along the UIM sequence, which creates a similar ubiquitin-binding surface on both sides of the α-helix.

There are other examples of double-sided UIMs, including human co-chaperone heat-shock protein DnaJ homologue (HSJ1), MAP kinase kinase kinase 1 (MAP3K1) and the endocytic regulators epidermal growth factor receptor substrate 15 (EPS15) and EPS15-related (EPS15R). Interestingly, Vps27, a yeast homologue of HRS, has two single-sided UIMs (see Supplementary information S2a (figure)), whereas mouse EPS15 also has tandem UIMs, but only one of them is double sided (see Supplementary

information S2b (figure)). It is conceivable that the double-sided UIM and/or tandem UIM arrangements evolved from copies of single-sided UIMs to provide additional levels of control.

Another example of double-sided ubiquitin binding is found in the ESCRT pathway. Golgi-localized, γ-ear-containing, ARF-binding proteins (GGAs) and target of MYB1 (TOM1) function as ESCRT-0-associated proteins[58,59]. The GGA and TOM (GAT) domains of GGA3 and TOM1 fold into a three-helix bundle to form two ubiquitin-binding surfaces: a high-affinity binding surface formed by the first and second helices (see Supplementary information S2c (figure))[58,60] and a low-affinity binding surface formed by the second and third helices[61,62]. As for UIMs, the dual ubiquitin recognition of these proteins might enhance their affinity for multiply ubiquitylated surface receptors.

Multisite interactions in DNA damage pathways. A prominent example among the various DNA damage pathways that are regulated by ubiquitin signals[63] is translesion DNA synthesis (TLS). TLS responds to damage-induced stalling at replication forks and provides a good example of the involvement of multiple UBDs in regulating the transcriptional response to DNA damage[64]. In the TLS pathway, DNA damage promotes monoubiquitylation on Lys164 of proliferating cell nuclear antigen (PCNA), a processivity factor that forms a sliding clamp around DNA. PCNA interacts weakly with numerous DNA polymerases, but monoubiquitylation of PCNA favours its preferential interaction with Y-family TLS polymerases, which possess the UBDs ubiquitin-binding motif (UBM) and UBZ. These error-prone polymerases can bypass the lesion, which would otherwise block DNA replication. Functional integrity of UBDs is essential for the proper localization of TLS polymerases to replication foci and for cellular survival following ultraviolet (UV) irradiation[38,65]. UBZ and UBM domains bind monoubiquitin with comparable affinities, but they interact through distinct surfaces: UBZ requires the Ile44-containing hydrophobic patch, whereas UBM binds independently of Ile44[22,38].

The presence of multiple functional UBMs in the mammalian polymerases reversionless 1 (REV1) and polymerase iota led to the hypothesis that these UBMs interact with multiple ubiquitylated components of replication foci. In accordance, mutation of either UBM of REV1 partially reduces its UV light-induced localization to stalled replication foci[66]. The effect is more severe in polymerase iota, as mutation of its UBM domains completely blocks its accumulation at replication foci during the S phase, and this result is independent of DNA damage[38]. The prominent role of the UBM domains does not seem to apply to all organisms, because in yeast cells UBM1 of Rev1 is not functional[66]. Swapping experiments have revealed that specific UBDs in host proteins are linked to distinct functional roles, but this is not the case for all UBDs. For instance, an exchange of the UBZ domain of Werner helicase-interacting protein 1 (WRNIP1), which is involved in the regulation of genome stability, for UBM1 of polymerase iota abolishes foci formation of WRNIP1 (REF. 67), suggesting that different UBDs have distinct functional roles in the context of full-size proteins.

Ubiquitin-binding motif (UBM). A structural ubiquitin-binding motif that is present in translesion DNA polymerases and is required for proper localization of these enzymes in nuclear replication foci.

Ubiquitin receptors at the proteasome

Although Lys48-linked ubiquitin chains are largely recognized as a major signal to target substrates for proteasomal degradation, new evidence indicates that an array of different ubiquitin chains can determine the kinetics and fidelity of substrate targeting to the proteasome. All of the observable ubiquitin linkages in HeLa cells, including Lys6, Lys11, Lys27, Lys33, Lys48 and Lys63, increase in abundance on inhibition of proteasomal degradation by the drug MG132 (REF. 68). In yeast, an increase was observed upon proteasome inhibition for all ubiquitin linkages except Lys63, which was shown to be the only linkage that does not have a role in proteasomal degradation[69]. This selection against Lys63 linkages in proteasome targeting is possibly due to its preferential binding to non-proteasomal UBDs, as discussed in previous sections, because Lys63-linked chains can signal for proteasomal degradation *in vitro*[45,70] and *in vivo*[71].

The only chain types that seem to be intrinsically impaired in their ability to stimulate substrate degradation are forked chains, in which a ubiquitin moiety is modified at two different Lys residues[45]. These data suggest that ubiquitin receptors at the proteasome do not select against chains of a certain linkage type and that local factors determine the presentation of ubiquitin chains to the 26S proteasome. The 26S proteasome contains a 20S core particle that carries out substrate proteolysis and is capped at one or both ends by a 19S regulatory particle. The regulatory particle prepares degradation substrates for passage through a narrow entry that leads to the catalytic centre of the core particle. It contains ubiquitin receptors for substrate recognition as well as DUBs and ATPases to deconjugate ubiquitin chains and unfold degradation substrates, respectively.

Proteasomal ubiquitin receptors. In *Saccharomyces cerevisiae*, there are five known ubiquitin receptors that can support protein degradation by the proteasome. Two of these receptors, Rpn10 (S5a in humans)[72] and Rpn13 (REF. 36), are 26S proteasome regulatory subunits, whereas the other three, UV excision repair protein Rad23, dominant suppressor of Kar1 (Dsk2), and DNA-damage inducible protein 1 (Ddi1), reversibly bind to the proteasome's regulatory particle. Orthologues of these proteins exist in higher eukaryotes, and some also have multiple paralogues. In yeast, none of these proteins is essential and strains with all five of these receptors deleted are viable, suggesting that other components can mediate substrate docking to the proteasome in their absence[36]. Some candidates have been implicated through crosslinking experiments[73,74]. In mice, RPN10 and RAD23 are essential[75,76], suggesting that they might have more specialized roles in higher eukaryotes.

RPN10 contains UIMs that bind monoubiquitin and ubiquitin chains[77]. The two UIMs of S5a adopt helical configurations and bind monoubiquitin independently owing to their separation by flexible linker regions[27] (see Supplementary information S2d (figure)). However, the two UIMs bind simultaneously to the two ubiquitin moieties in Lys48-linked diubiquitin for a markedly increased affinity[78]. S5a binds Lys48- and Lys63-linked chains equivalently but exhibits significantly weaker affinity for a mixture of Lys29- and Lys6-linked chains[42]. S5a also binds Lys11-linked ubiquitin chains, in accordance with their ability to effectively target substrates to the proteasome for degradation[79].

In contrast to S5a, RPN13 has only one ubiquitin-interacting surface, which occupies the same structural domain used to dock this receptor to the proteasome[35,36]. As discussed above, this domain folds into a PH domain, and three of its loops bind ubiquitin[35] (FIG. 4e). With the exception of the shorter Rpn13 protein from *S. cerevisiae*, RPN13 binds the DUB ubiquitin carboxy-terminal hydrolase isozyme L5 (UCHL5; also known as UCH37) and thereby recruits it to the proteasome's regulatory particle[80–82]. RPN13 seems to bridge substrate docking with ubiquitin chain deconjugation. Human RPN13 exhibits strong affinity for ubiquitin, even for monoubiquitin[36], and is therefore expected to bind to chains of all linkage type. It does show preference, however, for the proximal over the distal ubiquitin of Lys48-linked diubiquitin owing to favourable contacts with the linker region[35]. This preference is expected to render the distal ubiquitin moiety available for other interactions, such as that with UCH37. The binding of RPN13 to ubiquitin chains and UCH37 might simultaneously facilitate the deubiquitylating activity[80–82] of UCH37's distal end[83] by orienting the ubiquitin moieties in a configuration that is favourable to hydrolysis. Future experiments are needed to test this model and whether the ubiquitin-binding capacity of RPN13 contributes to its activation of UCH37.

Shuttling ubiquitin receptors. Proteins containing ubiquitin-like (UBL) and UBA domains can recruit ubiquitylated substrates to the proteasome for degradation[84–88]. Their UBL domain can bind to a scaffolding protein, Rpn1 (S2 in humans)[89], in the base of the proteasome regulatory particle, as well as to Rpn13 (REF. 36) and S5a[90–92], whereas their UBA domains bind to ubiquitin[93,94]. Depending on their cellular protein levels, UBL- and UBA-containing proteins can also inhibit the degradation of ubiquitylated substrates[88]. Such inhibition is thought to occur because UBA domains sequester ubiquitin chains to prevent deubiquitylation, which is a prerequisite to substrate hydrolysis by the 20S core particle of the proteasome[88,95,96].

The different UBL–UBA family members have different ubiquitin-binding preferences and modes. A human Rad23 orthologue, R23A, effectively sequesters chains of up to eight ubiquitin molecules[97] and, as discussed above, its C-terminal UBA domain sandwiches between the ubiquitin moieties of Lys48-linked diubiquitin, making close contact with the linker region that connects the two moieties[43] (FIG. 3a). These properties explain the ability of R23A to inhibit substrate deubiquitylation and are markedly different from the binding and activation of Uch37 by Rpn13. They presumably make Rad23 proteins better shuttling factors for passing substrates to the proteasome, as substrates are more likely to remain ubiquitylated during transport. There is evidence that certain proteasome substrates require specific ubiquitin receptors[85,88]. More studies are needed to define substrate selectivity requirements for specific receptors; however, specificity is expected to be particularly applicable in higher eukaryotes in which RAD23 proteins[76] and S5a[75] are essential for viability.

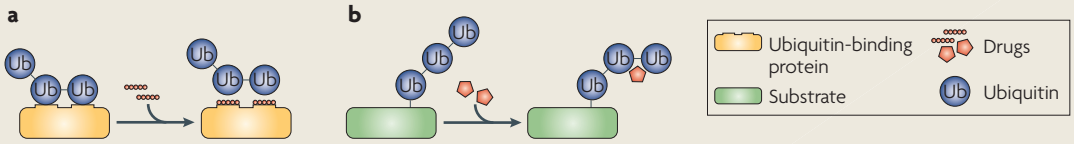
Ubiquitylation and ubiquitin binding

The functional relevance of the interplay between ubiquitylation and ubiquitin binding has been best demonstrated by the finding that multiple ubiquitin receptors in the endosome are monoubiquitylated and that this modification regulates their capacity to bind in *trans* to ubiquitylated cargo receptors[17,54,98–101]. This type of automodification, known as coupled monoubiquitylation, requires a functional UBD in the host protein to be autoubiquitylated[99–101]. Interestingly, many UBDs that bind to monoubiquitin can mediate such a modification in their natural proteins and can also act as transferable elements to mediate monoubiquitylation of newly formed chimeric proteins[102].

Mechanistically, there seem to be different ways in which UBD-containing proteins can undergo coupled monoubiquitylation. It can be achieved through direct binding of the UBD to ubiquitin-coupled E2 enzymes, thus being E3 independent[102]. Alternatively, an E3 ligase can bind to a UBD following its monoubiquitylation, as neural precursor cell expressed, developmentally downregulated 4 (NEDD4) does to the UIM of EPS15 (REF. 103). A UBL domain within the E3 ligase can also bind to the UBD to promote its ubiquitylation. For example, there is a prerequisite interaction between the UBL domain of RING-type E3 ligase parkin and the UIM of EPS15 for parkin-mediated ubiquitylation of EPS15 (REF. 98). The coupled monoubiquitylation might lead to an intramolecular interaction between ubiquitin and the UBD, which could in turn prevent interactions in *trans* with other ubiquitylated proteins[98,99,103]. Loss of such interactions can exert a negative-feedback control in regulatory networks that requires transient and consecutive interactions between the UBDs and their functional targets. In addition, the conformational change resulting from the intramolecular ubiquitin–UBD interaction might affect the functionality of other domains present in the protein, such as enzymatic activities or interactions with other cellular components.

Ubiquitylation of UBD-containing proteins might affect various other functions, such as the assembly of larger protein complexes or protein oligomerization. For example, ubiquitylation of the adaptor protein NEMO could modulate its oligomerization status. Lys285 and Lys309 of NEMO are linearly ubiquitylated by the LUBAC ligase[7]. The NEMO–linear diubiquitin crystal structure revealed that the non-covalent

Box 1 | **Targeting the ubiquitin–UBD interaction surface**



Selective binding between ubiquitin signals and their specific ubiquitin-binding domains (UBDs) transmits intracellular signals, controlling many biological functions that are often altered in human pathologies. Ubiquitin–UBD interactions are characterized by low affinity and dynamic exchange, and they often involve multiple surfaces in the protein complex. It is inherently difficult to use small chemical compounds to interfere with protein–protein interactions, especially those involving large binding surfaces. In the case of ubiquitin and UBDs, the interacting surface is generally flat and large. However, many mutagenesis studies indicate that the binding of ubiquitin to UBDs depends on a limited number of amino acid residues, which might represent 'hot spots'. Generally, good targets are surfaces with hot spots that can be covered by a drug-sized molecule (see the figure, part **a**). A problem with targeting ubiquitin, especially its commonly used Ile44-containing hydrophobic patch, is that its disruption will affect numerous UBD interactions and signalling pathways, leading to side effects. Ubistatins were the first chemical compounds identified to interfere with ubiquitin–UBD interactions, and these small molecules bind to ubiquitin chains and prevent their recognition by the 26S proteasome[106]. Clinical use of ubistatins has been hampered by poor bioavailability data; however, ubiquitin–UBD interactions can be efficiently disrupted by small compounds (see the figure, part **b**). Targeting specific UBD-containing effector proteins instead of ubiquitin is expected to allow the specific manipulation of distinct cellular processes. Progress in the structure-based drug design field, exemplified by the development of small peptidomimetic inhibitors that target proteins, such as inhibitors of apoptosis (IAPs) and B cell lymphoma 2 (BCL2), controlling apoptotic pathways in cancer cells (reviewed in REF. 105), indicates that it is pharmacologically possible to disrupt protein–protein interactions with small molecules. These approaches require new chemical synthesis strategies for 'peptidomimetic-like' compounds. Pharmacological development and usage of such modern therapeutics are promising.

interaction between the UBAN motif of NEMO and the linear ubiquitin chains is possible when NEMO is ubiquitylated at Lys285 (an in *trans* interaction; see Supplementary information S3 (figure)), but not when it is ubiquitylated at Lys309, which is at the heart of the ubiquitin-binding interface. Therefore, it is possible that these two ubiquitylation events are used to regulate oligomerization of ubiquitylated NEMO and, in turn, the production of large agglomerates for more efficient activation of IKK within such complexes. Future experiments are required to test whether such regulated oligomerization occurs and has any possible role in IKK activation.

Concluding remarks and future challenges

The role of ubiquitin as an important cellular signal has been established in many biological processes, much like phosphorylation. This knowledge has been rapidly expanding with the discovery of almost two dozen UBDs with distinct capacities to bind ubiquitin modifications and regulate different cellular processes. Structural analyses of ubiquitin–UBD interactions have become an essential foundation for better understanding specificity in ubiquitin networks. Structures of distinct ubiquitin modifications in complex with their respective UBDs have revealed dynamic changes in both ubiquitin signals and UBDs during their interactions. These complexes, although providing valuable data, have been composed largely of isolated UBDs and purified ubiquitin modifications (monoubiquitin or distinct ubiquitin chains) and thus lack an additional layer of information. This is particularly true for proteins that contain more than one UBD with different affinities and specificities for ubiquitin modifications (FIG. 4 and see Supplementary information S2 (figure)). Structural determination of both full-length proteins and multimeric ubiquitin-mediated complexes remains one of the biggest challenges. At present, there is no single structure of any protein conjugated with ubiquitin other than the structure of ubiquitin itself in different chain lengths.

The investigation of UBDs will surely expand and offer new surprises and opportunities. New classes of UBDs are continuously being reported, revealing new information not only about functionality but also about the mechanisms of intermolecular regulation. The finding that loops rather than secondary structural elements of a UBD can mediate ubiquitin binding indicates that distinct tertiary folds, which are not easily distinguishable as a modular domain, might create yet another set of ubiquitin-binding elements. Additionally, systems biology approaches are expected to expand our knowledge on the composition and behaviour of ubiquitin–UBD networks in various conditions. We have previously sought to define central hubs in ubiquitin–UBD networks and named them key nodes regulated by ubiquitin (KRUBs)[104]. It will be important to expand the list of KRUBs and to describe their properties and dynamics using *in vivo* experimental approaches and *in silico* simulations. Finally, the accumulated knowledge of ubiquitin–UBD interactions will also have an impact on pharmacological and medical applications (BOX 1). Several UBDs have now been linked to various human pathologies, including cancer and immune deficiencies, thus becoming interesting as putative targets for therapy[105].

Peptidomimetic inhibitor
A chemical or natural compound that mimics a peptide–peptide interaction through a non-peptide bond or structure.

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DATABASES
Protein Databank: http://www.rcsb.org/pdt
1AAR | 1D3Z | 1Q5W | 1ZO6 | 2D3G | 2DX5 | 2FIF | 2FUH | 2G45 | 2JF5 | 2JY6 | 2W9N | 2Z59
UniProtKB: http://www.uniprot.org
ABIN1 | CYLD | Ddi1 | Dsk2 | EAP45 | RABEX5 | RAD23A | Rpn10 | Rpn13 | UBCH5C | USP2 | WRNIP1

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