Linking K29-Ub chains to biology

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Linking K29-Ub chains to biology [OR] Piece 29 of the ubiquitin puzzle [OR] K29 no longer the missing link

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Different ubiquitin chain types serve as distinct cellular signals. A new synthetic antigen-binding fragment, sAB-K29, specifically recognises K29-linked diubiquitin and links K29 chains to proteotoxic stress and their accumulation in midbodies during mitosis.

Ubiquitylation is a versatile posttranslational modification that controls a large range of cellular processes in eukaryotic cells. Ubiquitin can be covalently linked to protein substrates and to other ubiquitin molecules to form ubiquitin chains. These ubiquitin chains are formed by the covalent attachment of the carboxyl terminus of one ubiquitin to Met1 or one of the seven Lys residues of another ubiquitin molecule thereby resulting in chains of eight different linkage types. Specific antibodies or binders have been developed against all linkage types excepting K29-linkages, which has hampered understanding of the biology of this linkage type. In this issue of Nature Chemical Biology, Yu et al. (*1*) develop a synthetic antigen binding fragment that binds to K29-linked diubiquitin with high affinity and specificity (sAB-K29). The authors use this powerful tool to identify potential roles for K29-linked ubiquitylation in proteotoxic stress responses and cell cycle regulation.

Despite being composed of identical building blocks, differently linked polyubiquitin chains adopt unique conformations creating distinctive interaction surfaces, which allow them to act as versatile signalling mediators. Antibodies and synthetic affinity reagents that specifically bind to a particular chain type have helped to untangle the many roles of different ubiquitin chain types. For instance, antibodies against K11 chains were instrumental in revealing its roles in the cell cycle and protein degradation (*2*). K29-linked ubiquitin is an abundant linkage type accounting for ~10% of all linkages in cells, but the lack of good tools to study this chain type has drastically limited our understanding to date (*3*, *4*). Previous efforts to isolate and study K29-linkages have relied on the Npl4 zinc finger (NZF) domain of the deubiquitinase TRABID (*5*) (**Fig 1A**). However, this K29 binder also recognizes K33-linked ubiquitin chains thus limiting its applicability in dissecting K29 specific effects.

In the present study, Yu et al. (*1*) screen a phage display library of synthetic antibody-binding fragments and identify sAB-K29, a specific binder with low nanomolar affinity to K29 chains. The crystal structure of sAB-K29 in complex with K29-diubiquitin reveals an unusual recognition mode that does not involve the commonly deployed hydrophobic I44 patch for binding. In contrast, sAB-K29 interacts with the I36 patch on the distal ubiquitin and recognizes the K29-linkage between the two ubiquitin moieties (**Fig 1B**). This binding mode differs from that of the NZF domain of TRABID. Importantly, sAB-K29 recognizes the isopeptide bond driving its specificity for K29-linked chains. Using several orthogonal assays, Yu et al. (*1*) validated that sAB-K29 binds to only K29 chains and none of the other linkage types. The authors also showed that the sAB-K29 antibody was applicable in many assays, including immunoblotting, immunoprecipitation, mass spectrometry and immunofluorescent imaging making this a versatile tool (**Fig 1C**).

With this specific K29-recognizing tool in hand, Yu et al. (*1*) set off to study the function of K29-linked chains. Cell cycle-related proteins colocalized with sAB-K29 around the midbody during telophase, suggesting the role of K29 chains in mitosis. Expression of the DUB TRABID to cleave K29 linkages led to cell cycle arrest. Since the levels of K29 chains did not change during the cell cycle and they showed no colocalization with the unfoldase VCP/p97 or the 20S proteasome, the authors posit non-degradative roles for K29 linkages in cell cycle regulation. Future studies will be needed to reveal how K29 chains enriched at the midbody function to mediate cell cycle progression.

In contrast to these non-degradative roles, Yu et al. (*1*) also find K29 chains to co-localize with stress granules, VCP and the proteasome, suggesting roles for K29 chains in protein degradation. Indeed, previous studies have associated K29 linkages with protein degradation in the ubiquitin fusion degradation (UFD) pathway in yeast (*6*) and more recently in PROTAC induced neo-substrate degradation by the E3 ligase TRIP12 (*7*). Interestingly, K29 linkages are commonly found to exist within polyubiquitin chains containing other linkage types (heterotypic chains) (*5*, *8*). For instance, K11-K48 heterotypic chains have been shown to function as efficient protein degradation signals and similarly it is thought that K29-K48 chains may function to mediate protein degradation. One possibility that reconciles these contrasting roles for K29 linkages is that homotypic K29 chains may have non-degradative roles whereas branched heterotypic K29 chains may facilitate degradation.

The development of sAB-K29 finally completes the reagent toolbox to specifically detect all eight homotypic ubiquitin chain types. The ascription of K29-linked ubiquitylation to stress responses and cell cycle regulation fills in missing gaps in our understanding of this abundant yet enigmatic posttranslational modification. While these new antibodies will be valuable tools to explore the biology of K29 chains, new challenges lie ahead already in the ever-expanding ubiquitin signalling field, for example, in the form of heterotypic ubiquitin chains containing mixed and branched

linkages and the recent emergence of non-proteinaceous ubiquitylation (*9*). It will be exciting to see future studies, using the now complete set of available tools to detect ubiquitin chains, to reveal what proteins are modified by K29-linked ubiquitin and to gain insights into the complexity of heterotypic, branched ubiquitin chains containing K29-linkages.

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Competing interests
The authors declare no competing interests

Figure:

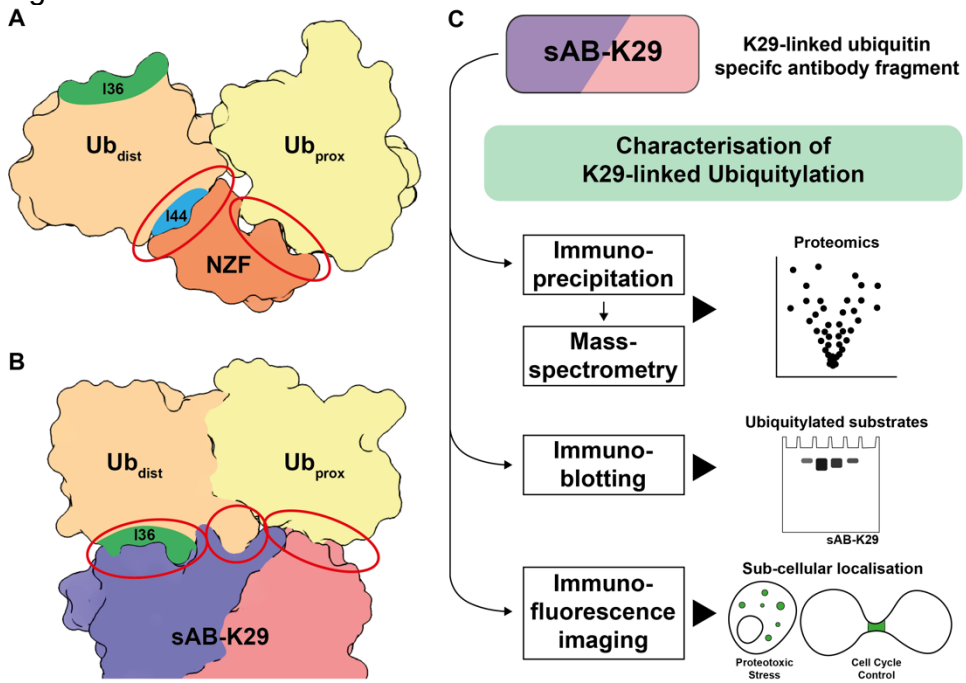

Fig. 1 | **New tool to study K29-linked ubiquitylation**. A) Clipped surface model of TRABID NZF domain bound to K29-linked diubiquitin (PDB 4S1Z). Distal and proximal ubiquitin (Ub_{dist}, Ub_{prox}) in orange and yellow, respectively, with highlighted hydrophobic I36 (green) and I44 (blue) patches on Ub_{dist}. B) Clipped surface model of sAB-K29 antigen-binding site in complex with K29-linked diubiquitin (PDB 7KEO). Colour labels as in previous panel, heavy and light chain of sAB-K29 in purple and pink. C) Flow chart of methods and experiments enabled by the new sAB-K29 tool as reported by Yu et al. (2021).