Photoaffinity Labelling

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Chemical Synthesis of Diubiquitin-Based Photoaffinity Probes for Selectively Profiling Ubiquitin-Binding Proteins

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Abstract: *Biochemical studies of cellular processes involving polyubiquitin have gained increasing attention. More tools are needed to identify ubiquitin (Ub)-binding proteins. We report diazirine-based photoaffinity probes that can capture Ub-binding proteins in cell lysates, and show that diazirines are preferable to aryl azides as the photo-crosslinking group, since they decrease non-selective capture. Photoaffinity probes containing at least two Ub units were required to effectively capture Ub-binding proteins. Different capture selectivity was observed for probes containing diubiquitin moieties with different types of linkages, thus indicating the potential to develop linkage-dependent probes for selectively profiling Ub-binding proteins under various cellular conditions.*

As one of the most important posttranslational modifications (PTMs) in eukaryotes, ubiquitination is involved in a wide range of cellular processes.[1] This modification exhibits higher complexity than other PTMs (e.g., phosphorylation) because eight different types of polyubiquitin (polyUb) chains are formed, through Met1, Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, or Lys63 linkages.[2] Previous studies have established that all eight polyUbs are present in cells and they show functional variations.[3] For instance, Lys48-linked polyUbs are signals for proteasomal degradation, whereas Lys63-linked polyUbs are involved in signal transduction and DNA repair. The distinct cellular signals of different polyUbs are controlled by the recognition and decoding of polyUbs by ubiquitin (Ub)-binding proteins containing ubiquitin-binding domains (UBDs).[4]

The identification of Ub-binding proteins, especially those that can selectively recognize different polyUb linkages, is fundamental to studying the cellular roles and regulatory mechanisms of ubiquitination. For this purpose, bioinformatics,[5] yeast two-hybrid assays,[6] and affinity pull-down technology[7] have been used, which has led to the capture and characterization of approximately 150 Ub-binding proteins.[8,9] For instance, Pickart et al. used a K48-linked Ub_4 aryl-azide-based probe to identify a novel Ub-binding site in the proteasome.[10] Given that there are different UBDs that specifically recognize the eight different types of polyUb chains, we reasoned that incorporating photoaffinity groups into different Ub chains may enable the identification of specific UBDs.

Herein, we report diazirine-based photoaffinity probes to capture Ub-binding proteins. These probes are tailor-designed proteins generated through chemical protein synthesis. Our work was inspired by the recent success of using photoaffinity probes to capture proteins bound to other types of PTMs.[11] We found that photoaffinity probes with at least two Ub units are required to effectively capture Ub-binding proteins, and that diazirine is a more effective photo-crosslinking group than aryl azide for capturing Ub-binding proteins. Interestingly, we observed distinct capturing performance for probes containing different diUbs, thus suggesting the need to develop linkage-dependent probes to examine or monitor Ub-binding proteins.

We initially designed aryl-azide-based photoaffinity probes containing one Ub (**1**), Lys48-linked diUb (**2**), and Lys63-linked diUb (**3**; Figure 1A). Because the binding between Ub and UBDs usually involves the Ile44 hydrophobic patch of Ub,[12] we put a photo-crosslinking phenyl azide group at the Ala46 that neighbors Ile44. An affinity handle (biotin) was placed at the C terminus of the probes to enrich photo-crosslinked products. Probes containing both isopeptide bonds and photo-crosslinking groups are difficult to prepare through either expression or enzymatic constitution.[13] As a result, we carried out protein total synthesis, which allows exquisite control at the atomic level.[14] For example, to prepare **3**, we divided it into three segments: **4**, **5**, and **6** (Figure 2A). To construct the isopeptide bond, a trifluoroacetic acid (TFA)-cleavable 1-(2,4-dimethoxyphenyl)-2-mercaptoethyl auxiliary (Aux) was introduced into **6** (see the Supporting Information).[15] Both Ala46 residues from the two Ub units were mutated to Cys for convenience of ligation, as well as the subsequent incorporation of photo-crosslinking groups.

Peptide segments **4**–**6** were synthesized using Fmoc-based solid-phase peptide synthesis (SPPS, Fmoc = 9-fluorenylmethoxy-carbonyl) and purified using reversed-phase high-performance liquid chromatography (RP-HPLC). Aux-assisted ligation between **5** (1.2 equiv) and **6** (1.0 equiv) was performed by using hydrazide-based native chemical ligation (NCL).[16] This reaction was completed in approximately 12 h

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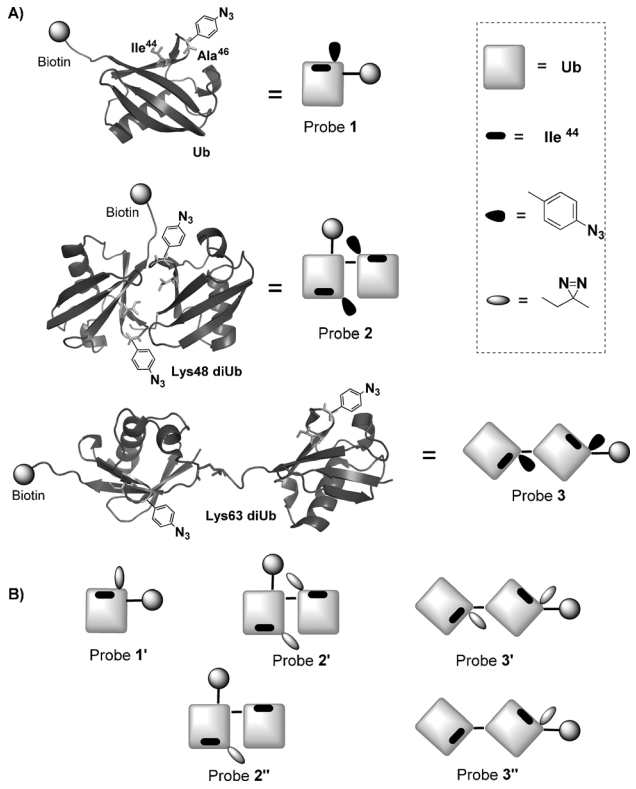


Figure 1. Photoaffinity probes with crosslinking groups attached to Ala 46. A) Phenyl-azide-based ubiquitin probes **1**, **2**, and **3**. B) The diazirine-based ubiquitin probes **1′**, **2′**, **3′**, **2″**, and **3″**.

with a 49% yield of isolated product (Figure 2B). The product (**7**) was treated with TFA to remove Aux, and this was followed by AgOAc treatment to remove Acm, which led to intermediate **8** with a yield of 79%.[17] Subsequently, ligation between **8** (1.0 equiv) and **4** (2.1 equiv) was performed to generate **9**. Selective modification of the Cys residues of **9** was conducted with 1-(4-azidophenyl)-2-bromoethan-1-one to produce probe **3** with an overall yield of 14%.[18] The identity and purity of **3** were characterized through analytical RP-HPLC and electrospray-ionization mass spectrometry (ESI-MS; Figure 2C,E and the Supporting Information). Circular dichroism (CD) spectroscopy was used to characterize the secondary structure of **3** (Figure 2G), and the probes showed absorption at both 208 and 226 nm. These spectra showed very similar patterns to that of monoubiquitin (monoUb), thus indicating that the Ub units in **3** retain the correct globular fold.[19]

We next examined the ability of **1**, **2**, and **3** to capture model Ub-binding proteins. The tandem ubiquitin-interacting motif (tUIM) in human protein Rap80 (residues 80–121) was chosen as the first target. Cohen et al. have shown that tUIM preferentially binds to Lys63-linked diUb (binding constant $K_d = 17.6\ \mu M$) over Lys48-linked diUb ($K_d = 157\ \mu M$) or monoUb ($K_d = 520\ \mu M$).[20] We therefore expected **3** to selectively capture more tUIM than **1** or **2**. To test this hypothesis, we prepared tUIM through chemical synthesis. After folding the synthetic tUIM, we mixed tUIM (2.0 equiv, 5.0 μM) with **1**, **2**, or **3** (1.0 equiv, 2.5 μM). The mixtures were incubated on ice for 1.5 h and then exposed to 254 nm UV light for the indicated period of time (1 or 5 min). After streptavidin enrichment, the mixtures were analyzed by sodium dodecyl sulfate-polyacrylamide polyacrylamide gel electrophoresis (SDS-PAGE). Unexpectedly, **1**, **2**, and **3** crosslinked to tUIM with almost the same efficiency (Figure 3A). Furthermore, with an extended irradiation time from 1 to 5 min, **2** and **3** crosslinked with two tUIM molecules each, a result that is not consistent with a previous report indicating that only one tUIM can bind K63-linked diUb.[20] To reconcile this discrepancy, we analyzed gel bands corresponding to the crosslinked products by using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). A crosslinked peptide fragment containing Cys 121 of tUIM was observed for all of the above experiments (Figure 3C), thus indicating that Cys 121 might play an essential role in all of the crosslinking products. A previous report[21] revealed that the nitrene intermediate produced after the loss of N_2 from an aryl azide tends to photoisomerize to dehydroazepine, which can react with nucleophiles (Figure 3D). The observation that **2** and **3** crosslink to two tUIM molecules through Cys indicates that aryl-azide-based crosslinking reactions can occur through nucleophilic addition.

To further test the aryl-azide-based probes, we chose the ubiquitin-associated 2 domain (UBA2) from the human homologue of the yeast protein Rad23 (residues 315–363) as the second target. Previous studies have shown that UBA2 preferentially binds Lys48-linked diUb ($K_d = 11.5\ \mu M$) over Lys63-linked diUb ($K_d = 143.6\ \mu M$) or monoUb ($K_d = 540\ \mu M$).[23] In our study, UBA2 was prepared through chemical synthesis. After folding, synthetic UBA2 (2.0 equiv, 5.0 μM) was reacted with **1**, **2**, or **3** (1.0 equiv, 2.5 μM) under the same photo-crosslinking conditions as described above for tUIM. The corresponding reaction mixtures were resolved with SDS-PAGE, and we found that **2** and **3** crosslinked to UBA2 with almost the same efficiency (Figure 3B). Therefore, we concluded that aryl azide is not a suitable crosslinking group for developing photoaffinity probes to selectively capture Ub-binding proteins.

To avoid non-selective crosslinking, the alternative photoaffinity group diazirine was considered.[22] The diazirine-based photoaffinity probes **1′**, **2′**, and **3′** (Figure 3B) were obtained in a similar manner to **1**, **2**, and **3** through protein chemical synthesis (Figure 2A,C,F and the Supporting Information). Probes **1′**, **2′**, and **3′** were used for the crosslinking reaction. Using SDS-PAGE to analyze the photo-crosslinking reactions with Rap80 tUIM, we observed that **1′**, which contains only one Ub unit, did not crosslink with tUIM (Figure 4A left). For probes containing two Ub units, only **3′** specifically crosslinked with tUIM (Figure 4A, left). Importantly, when we added increasing doses of Lys63-linked diUb into the photo-crosslinking mixtures of **3′** with the tUIM, we observed a dose-dependent decrease in crosslinking product (Figure 4C). This result indicates that Lys63-diUb competes with tUIM labeling by **3′** (2.5 μM) with $IC_{50} = 57.5\ \mu M$, a value that matches the reported 17.6 μM binding constant (K_d).[20] In addition, we tested the photo-crosslinking efficiency of **3′** (2.5 μM) with tUIM and found that tUIM crosslinks with **3′** in a dose-dependent manner without observing any cross-link-

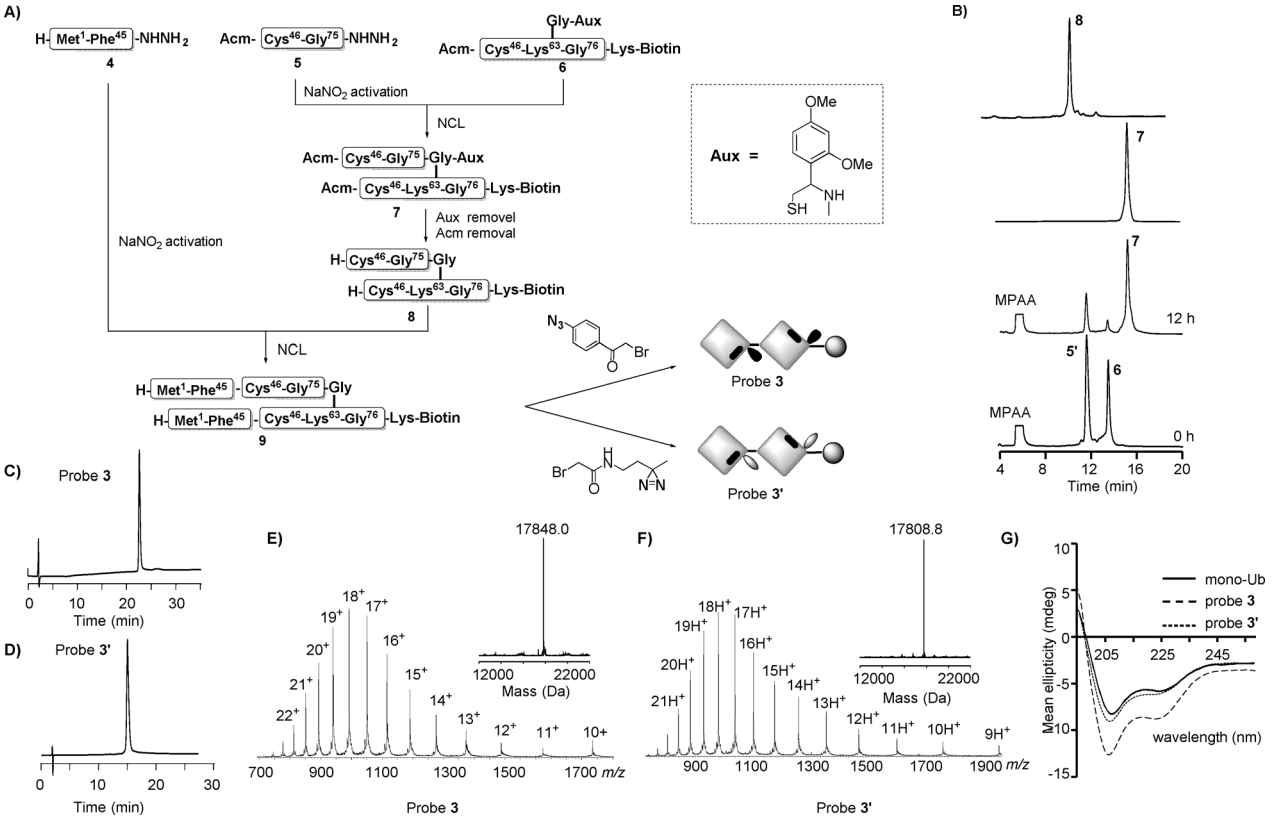


Figure 2. Chemical synthesis of probes **3** and **3′**. A) The synthesis route. Acm = acetamidomethyl. B) Auxiliary-assisted ligation monitored by analytical RP-HPLC. **5′** is the 4-mercaptophenylacetic acid (MPAA) thioester Ub [Met 1-Phe 45]-MPAA. C–D) Analytical RP-HPLC chromatograms of purified **3** and **3′**. E–F) ESI-MS of **3** and **3′**. G) CD spectra of **3** and **3′** compared with monoUb.

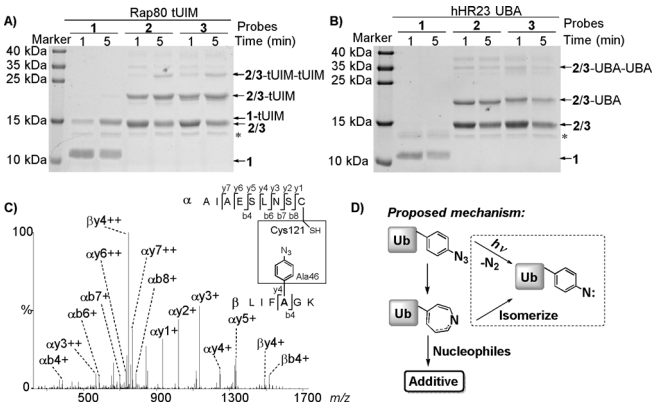


Figure 3. A, B) SDS-PAGE analysis of the photo-crosslinking reactions with Rap80 tUIM or hHR23a UBA2 using probes **1**, **2**, and **3**. * indicates monomeric streptavidin. C) LC-MS/MS analysis of the cross-linked peptide fragments of **1**, **2**, and **3** with tUIM. D) Proposed mechanism of unwanted phenyl azide crosslinking. Proteins were separated with 15% SDS-PAGE and analyzed through Coomassie blue staining.

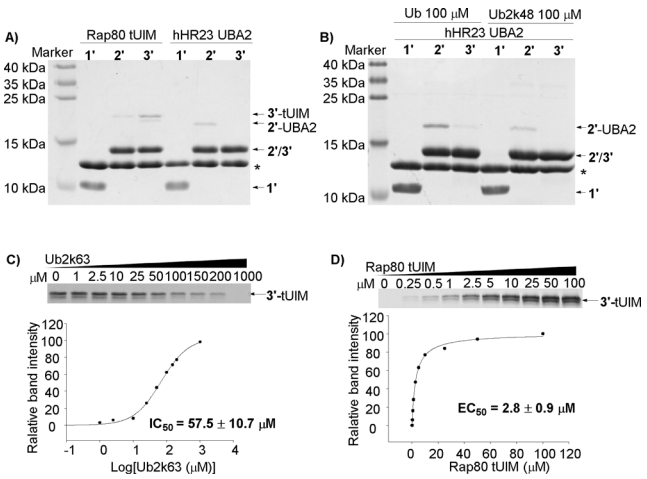


Figure 4. A) Photo-crosslinking reactions of **1′**, **2′** and **3′** with Rap80 tUIM or hHR23a UBA2. B) Photo-crosslinking of the probes with UBA2 was selectively inhibited by Lys 48-diUb but was unaffected by mono-Ub. Proteins were separated by 15% SDS-PAGE and analyzed through Coomassie staining. * indicates monomeric streptavidin. C) Lys 63-diUb inhibited photo-crosslinking of **3′** with Rap80 tUIM. D) Concentration-dependent labeling of tUIM by **3′**.

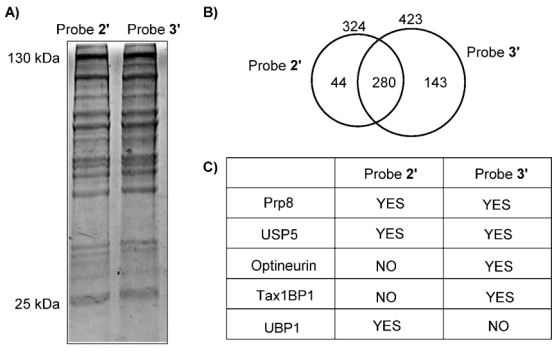
ing of **3′** with two tUIM molecules. The labeling saturated at approximately 10 μM tUIM ($EC_{50} = 2.8$ μM) (Figure 4 D), thus indicating that the introduction of photoaffinity groups did not substantially affect Lys 63-diUb binding to tUIM. Additionally, no crosslinking was detected between **3′** and Lys 63-linked diUb (50- to 100-fold more than tUIM) in this experiment. These observations strongly suggest that the crosslinking of **3′** exhibits high protein selectivity. Probes **2″** and **3″** (Figure 1 B, Figure S31 in the Supporting Information),

which have only one photoaffinity label, were also chemically synthesized for the photo-crosslinking reactions, and the photo-crosslinking capture efficiency and specificity of this probe set were similar to those for the probes with two photoaffinity labels (Figure S31). Because the synthesis yields of **2″** and **3″** were relatively low (7% for **2″** and 5% for **3″**, Figure S17), **2′** and **3′** were chosen for the following experiments.

To further confirm the crosslinking selectivity of the diazirine-based photoaffinity probes, UBA2 was tested. We found that UBA2 was only captured by probe **2′**, and no crosslinking was observed for **1′** or **3′** (Figure 4A, right). The labeling of UBA2 is subject to competition by native Lys48-diUb. Importantly, this competition was specific, because the presence of monoUb did not interfere with labeling (Figure 4B), thus verifying that the diUb probes exhibit linkage-dependent selectivity for Ub-binding proteins.

Finally, we examined whether diazirine-based probes could be used to profile Ub-binding proteins in real proteomes. Probes **2′** and **3′** (2.5 μM) were added to 293F cell lysates (2 mg mL^{-1}). The samples were irradiated with 365 nm UV light for 10 min, and streptavidin-coupled beads were used to enrich for the putative targets. After resolution by SDS-PAGE (Figure 5A) and in-gel digestion with trypsin, the proteins enriched by streptavidin were characterized by LC–MS/MS. After subtracting the control hits (irradiated cell lysates without any probe) from the samples, we identified 324 proteins bound to **2′** and 423 proteins bound to **3′** (Figure 5B and the Supporting Information). Analysis revealed that 280 proteins were captured by both **2′** and **3′**, including many known Ub-binding proteins, as well as Ub-related enzymes such as RABGEF1, Prp8, HDAC6, USP5, and USP13.[4] Furthermore, 143 proteins were captured by only **3′** but not **2′** (Figure 5C), thus suggesting a binding preference for Lys63-linked polyUbs, as has been validated by studies of Rap80, Optineurin, and Tax1BP1, which are known Lys63-polyUb binders.[20,24] Moreover, we identified some proteins (e.g., GGA3) that have not been well characterized but may bind Lys63 polyUbs. There were fewer proteins (44) captured only by **2′** and not **3′**. Some of these 44 proteins are known to bind Lys48 polyUbs, such as the deubiquitinase UBP1, which is known to modulate Lys48 polyUb-modified P53 (Figure 5C).[25] The above results indicate that the diazirine photoaffinity probes selectively crosslink with Ub-binding proteins.

In summary, photoaffinity probes with oligoubiquitin skeletons were developed as new tools for the proteomic identification of Ub-binding proteins. We have demonstrated that these probes can be efficiently prepared through total chemical synthesis and can be precisely functionalized with isopeptide linkages and photo-crosslinking groups. An important finding of our work is that probes with different diUbs exhibit altered selectivity for capturing proteins, thus indicating the potential for linkage-dependent Ub probes that could be applied to identifying new Ub-binding proteins for subsequent biochemical and functional studies.



	Probe 2'	Probe 3'
Prp8	YES	YES
USP5	YES	YES
Optineurin	NO	YES
Tax1BP1	NO	YES
UBP1	YES	NO

Figure 5. A) SDS-PAGE analysis of proteins enriched by **2′** and **3′**. B) Comparative analysis. The Venn diagram shows that 44 and 143 proteins were captured by **2′** and **3′**, respectively, and 280 proteins were captured by both **2′** and **3′**. C) A representative list of known ubiquitin-binding proteins.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: native chemical ligation · photoaffinity labelling · protein modifications · protein synthesis · ubiquitination

[1] D. Mukhopadhyay, H. Riezman, *Science* **2007**, *315*, 201–205.
[2] a) D. Komander, M. Rape, *Annu. Rev. Biochem.* **2012**, *81*, 203–229; b) T. Schneider, D. Schneider, D. Rösner, S. Malhotra, F. Mortensen, T. U. Mayer, M. Scheffner, A. Marx, *Angew. Chem. Int. Ed.* **2014**, *53*, 12925–12929; *Angew. Chem.* **2014**, *126*, 13139–13143.
[3] a) W. Kim, E. J. Bennett, E. L. Huttlin, A. Guo, J. Li, A. Possemato, M. E. Sowa, R. Rad, J. Rush, M. J. Comb, J. W. Harper, S. P. Gygi, *Mol. Cell* **2011**, *44*, 325–340; b) P. Xu, D. M. Duong, N. T. Seyfried, D. Cheng, Y. Xie, J. Robert, J. Rush, M. Hochstrasser, D. Finley, J. Peng, *Cell* **2009**, *137*, 133–145.
[4] a) I. Dikic, S. Wakatsuki, K. J. Walters, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 659–671; b) K. Husnjak, I. Dikic, *Annu. Rev. Biochem.* **2012**, *81*, 291–322.
[5] K. Hofmann, P. Bucher, *Trends Biochem. Sci.* **1996**, *21*, 172–173.
[6] a) S. C. Shih, G. Prag, S. A. Francis, M. A. Sutanto, J. H. Hurley, L. Hicke, *EMBO J.* **2003**, *22*, 1273–1281; b) P. M. Scott, P. S. Bilodeau, O. Zhdankina, S. C. Winistorfer, M. J. Hauglund, M. M. Allaman, W. R. Kearney, A. D. Robertson, A. L. Boman, R. C. Piper, *Nat. Cell Biol.* **2004**, *6*, 252–259.
[7] a) D. Seigneurin-Berny, A. Verdel, S. Curtet, C. Lemercier, J. Garin, S. Rousseaux, S. Khochbin, *Mol. Cell. Biol.* **2001**, *21*, 8035–8044; b) Y. H. Chiu, Q. M. Sun, Z. J. Chen, *Mol. Cell* **2007**, *27*, 1014–1023.
[8] L. Hicke, H. L. Schubert, C. P. Hill, *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 610–621.

[9] A. Peisley, B. Wu, H. Xu, Z. J. Chen, S. Hur, *Nature* **2014**, *509*, 110 – 114.
[10] Y. A. Lam, T. G. Lawson, M. Velayutham, J. L. Zweler, C. M. Pickart, *Nature* **2002**, *416*, 763 – 767.
[11] a) X. Li, T. M. Kapoor, *J. Am. Chem. Soc.* **2010**, *132*, 2504 – 2505; b) R. E. Kleiner, P. Verma, K. R. Molloy, B. T. Chait, T. M. Kapoor, *Nat. Chem. Biol.* **2015**, *11*, 807 – 814; c) Z. Liu, T. Yang, X. Li, T. Peng, H. C. Hang, X. D. Li, *Angew. Chem. Int. Ed.* **2015**, *54*, 1149 – 1152; *Angew. Chem.* **2015**, *127*, 1165 – 1168.
[12] K. Husnjak, I. Dikic, *Annu. Rev. Biochem.* **2012**, *81*, 291 – 322.
[13] a) K. S. A. Kumar, L. Spasser, L. A. Erlich, S. N. Bavikar, A. Brik, *Angew. Chem. Int. Ed.* **2010**, *49*, 9126 – 9131; *Angew. Chem.* **2010**, *122*, 9312 – 9317; b) F. El Oualid, R. Merkx, R. Ekkebus, D. S. Hameed, J. J. Smit, A. de Jong, H. Hilkmann, T. K. Sixma, H. Ovaa, *Angew. Chem. Int. Ed.* **2010**, *49*, 10149 – 10153; *Angew. Chem.* **2010**, *122*, 10347 – 10351; c) R. K. Singh, A. Sundar, D. Fushman, *Angew. Chem. Int. Ed.* **2014**, *53*, 6120 – 6125; *Angew. Chem.* **2014**, *126*, 6234 – 6239; d) S. Gao, M. Pan, Y. Zheng, Y. Huang, Q. Zheng, D. Sun, L. Lu, X. Tan, X. Tan, H. Lan, J. Wang, T. Wang, J. Wang, L. Liu, *J. Am. Chem. Soc.* **2016**, *138*, 14497 – 14502.
[14] a) P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776 – 779; b) S. B. H. Kent, *Chem. Soc. Rev.* **2009**, *38*, 338 – 351; c) M. Seenaiah, M. Jbara, S. M. Mali, A. Brik, *Angew. Chem. Int. Ed.* **2015**, *54*, 12374 – 12378; *Angew. Chem.* **2015**, *127*, 12551 – 12555; d) X. Chen, S. Tang, J. S. Zheng, R. Zhao, Z. P. Wang, W. Shao, H.-N. Chang, J. Y. Cheng, H. Zhao, L. Liu, H. Qi, *Nat. Commun.* **2015**, *6*, 7220.
[15] a) D. Macmillan, D. W. Anderson, *Org. Lett.* **2004**, *6*, 4659 – 4662; b) M. Pan, S. Gao, Y. Zheng, X. Tan, H. Lan, X. Tan, D. Sun, L. Lu, T. Wang, Q. Zheng, Y. Huang, J. Wang, L. Liu, *J. Am. Chem. Soc.* **2016**, *138*, 7429 – 7435; c) J. Li, Q. He, Y. Liu, S. Liu, S. Tang, C. Li, D. Sun, X. Li, M. Zhou, P. Zhu, G. Bi, Z. Zhou, J.-S. Zheng, C. Tian, *ChemBioChem* **2016**, DOI: 10.1002/cbic.201600551.
[16] a) G.-M. Fang, Y.-M. Li, F. Shen, Y.-C. Huang, J.-B. Li, Y. Lin, H.-K. Cui, L. Liu, *Angew. Chem. Int. Ed.* **2011**, *50*, 7645 – 7649; *Angew. Chem.* **2011**, *123*, 7787 – 7791; b) J.-S. Zheng, S. Tang, Y.-K. Qi, Z.-P. Wang, L. Liu, *Nat. Protoc.* **2013**, *8*, 2483 – 2495; c) J. S. Zheng, S. Tang, Y. C. Huang, L. Liu, *Acc. Chem. Res.* **2013**, *46*, 2475 – 2484; d) Y. C. Huang, L. Liu, *Sci. China Chem.* **2015**, *58*, 1779; e) Y. C. Huang, G. M. Fang, L. Liu, *Nat. Sci. Rev.* **2016**, *3*, 107.
[17] a) B. L. Pentelute, S. B. H. Kent, *Org. Lett.* **2007**, *9*, 687 – 690; b) S. Tang, Z. Wan, Y. Gao, J. S. Zheng, J. Wang, Y. Y. Si, X. Chen, H. Qi, L. Liu, W. Liu, *Chem. Sci.* **2016**, *7*, 1891 – 1895.
[18] Y.-K. Qi, H.-N. Chang, K.-M. Pan, C. Tian, J.-S. Zheng, *Chem. Commun.* **2015**, *51*, 14632 – 14635.
[19] K. S. A. Kumar, S. N. Bavikar, L. Spasser, T. Moyal, S. Ohayon, A. Brik, *Angew. Chem. Int. Ed.* **2011**, *50*, 6137 – 6141; *Angew. Chem.* **2011**, *123*, 6261 – 6265.
[20] J. J. Sims, R. E. Cohen, *Mol. Cell* **2009**, *33*, 775 – 783.
[21] Y. Tanaka, M. R. Bond, J. J. Kohler, *Mol. BioSyst.* **2008**, *4*, 473 – 480.
[22] a) L. Dubinsky, B. P. Kron, M. M. Meijler, *Bioorg. Med. Chem.* **2012**, *20*, 554 – 570; b) T. Yang, X. M. Li, X. Bao, Y. M. E. Fung, X. D. Li, *Nat. Chem. Biol.* **2016**, *12*, 70 – 72.
[23] a) S. Rassi, R. Varadan, D. Fushman, C. M. Pickart, *Nat. Struct. Mol. Biol.* **2005**, *12*, 708 – 714; b) N. Haj-Yahya, M. H. Yahya, C. A. Castaneda, L. Spasser, H. P. Hemantha, M. Jbara, M. Penner, A. Ciechanover, D. Fushman, A. Brik, *Angew. Chem. Int. Ed.* **2013**, *52*, 11149 – 11153; *Angew. Chem.* **2013**, *125*, 11355 – 11359.
[24] a) L. Verstrepen, K. Verhelst, I. Carpentier, R. Beyaert, *Trends Biochem. Sci.* **2011**, *36*, 347 – 354; b) G. Zhu, C. J. Wu, Y. Zhao, J. D. Ashwell, *Curr. Biol.* **2007**, *17*, 1438 – 1443.
[25] S. Glockzin, F. X. Ogi, A. Hengstermann, M. Scheffner, C. Blattner, *Mol. Cell. Biol.* **2003**, *23*, 8960 – 8969.

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Communications

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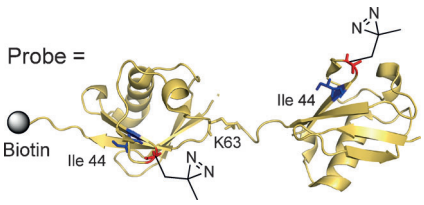
J. Liang, L. Zhang, X.-L. Tan, Y.-K. Qi, S. Feng, H. Deng, Y. Yan, J.-S. Zheng, L. Liu, C.-L. Tian* _____ ■■■–■■■

Chemical Synthesis of Diubiquitin-Based Photoaffinity Probes for Selectively Profiling Ubiquitin-Binding Proteins



Spoilt for choice: Diazirine-based photoaffinity probes for ubiquitin (Ub)-binding proteins based on K48-and K63-linked diubiquitin were developed. Diazirines were shown to be preferable to aryl azides as the photo-crosslinking group, since they decrease non-selective capture. Different capture selectivity was observed for the probes with different types of linkage, thus indicating the potential to develop linkage-dependent probes for selectively profiling Ub-binding proteins.