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Selective Triazenation Reaction (STaR) of Secondary Amines for Tagging Monomethyl Lysine Post-Translational Modifications

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Abstract: *Lysine monomethylation (Kme) is an impactful post-translational modification (PTM) responsible for regulating biological processes and implicated in diseases, thus there is great interest in identifying these methylation marks globally. However, the progress in this area has been challenging because the addition of a small methyl group on lysine leads to negligible change in the bulk, charge, and hydrophobicity. Herein, we report an empowering chemical technology selective triazenation reaction, which we term "STaR", of secondary amines using arene diazonium salts to achieve highly selective, rapid, and robust tagging of Kme peptides from a complex mixture under biocompatible conditions. Although the resulting triazene-linkage with Kme is stable, we highlight the efficient decoupling of the triazene-conjugate to afford unmodified starting components under mild conditions when desired. Our work establishes a unique chemoselective, traceless bioconjugation strategy for the selective enrichment of Kme PTMs.*

Introduction

Lysine methylation is one of the most important post-translational modifications (PTMs) of proteins because it regulates various biological processes including cell growth, division, gene expression, and DNA/RNA binding.[1–5] Due to lysine's unique structure, it has the ability to undergo mono, di, and tri methylation, and different levels of methylation give rise to different functions and localization within a cell.[6] PTMs regulate cellular processes by influencing the interactions of proteins with other proteins, nucleic acids, and lipids. We focused our attention particularly on monomethyl lysine (Kme) PTM, which occurs on both histones and non-histone proteins.[7–11] Kme PTMs have been implicated not only in transcriptional activation and silencing but the aberrant PTM levels have been linked to numerous diseases and disorders such as heart disease, cancer, and diabetes.[12–19] Accordingly, there is a great interest in mapping out the global role of Kme PTMs in development and diseases. However, tools to detect Kme PTMs are limited compared to other PTMs[20–22] because the addition of a methyl group leads to a negligible alteration in protein's or peptide's physicochemical properties.[23,24] The first challenge is that monomethylation of lysine does not add a substantial steric bulk and significantly change their hydrophobicity.[25] Second, the lysine monomethylation does not neutralize their positive charge (Figure 1a).[25] Moreover, the methyl group is too small to be captured by specific antibodies. Thus, it is very difficult to develop a highly efficient enrichment strategy.[26]

The current methods for detecting Kme PTMs involve the use of antibodies[25,27] and methyl binding domains.[28,29] However, these affinity reagents suffer from serious drawbacks and are unable to completely detect all the Kme sites because they are unable to distinguish between different lysine methylation states (mono, di- or tri-), adjacent PTMs can negatively affect antibody recognition, cross-reactivity with off target antigens, and batch to batch irreproducibility.[30–32] Moreover, they cannot be used to identify unknown Kme PTMs due to their high sequence specificity. Recently, small molecules and peptides mimicking chromodomains have been reported for identifying trimethyl lysine (Kme3) PTMs but tools to selectively detect Kme are still lacking.[33,34] Another widely used approach for detecting Kme utilizes mass spectrometry (MS). Although powerful but MS analysis is expensive, time-consuming and can be complicated by analytical challenges, including the low natural abundance of Kme PTMs in complex mixtures. Also, the change in the mass by monomethylation is identical to the substitutions of some amino acids thus leading to the false identification.[25,35] Together, none of these methods are suitable for selective tagging of Kme in a pan-specific manner due to the lack of empowering chemical technology.

A game changer for studying lysine monomethylation would be the discovery of novel chemoselective bioconjugation reaction for secondary amines[36] thus capable of tagging and identification of Kme PTMs in a pan-specific manner, independent of the peptide sequence and presence of other reactive functional groups. As such, we sought to pursue triazenation for selective tagging of Kme. Many coupling methods exist that bring chemical units together, but the methods that can be reversed easily to generate the unchanged units are lacking. We report a method, termed selective triazenation reaction ("STaR"), that allows chemoselective Kme tagging and enrichment from a complex mixture, followed by chemically triggered decoupling to

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Supporting information, including materials, instruments, reaction procedures, STaR, chemoselectivity studies, stability studies, pan-specificity studies, enrichment studies, traceless cleavage and products characterization by HRMS, HPLC, and NMR, and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.202013997.

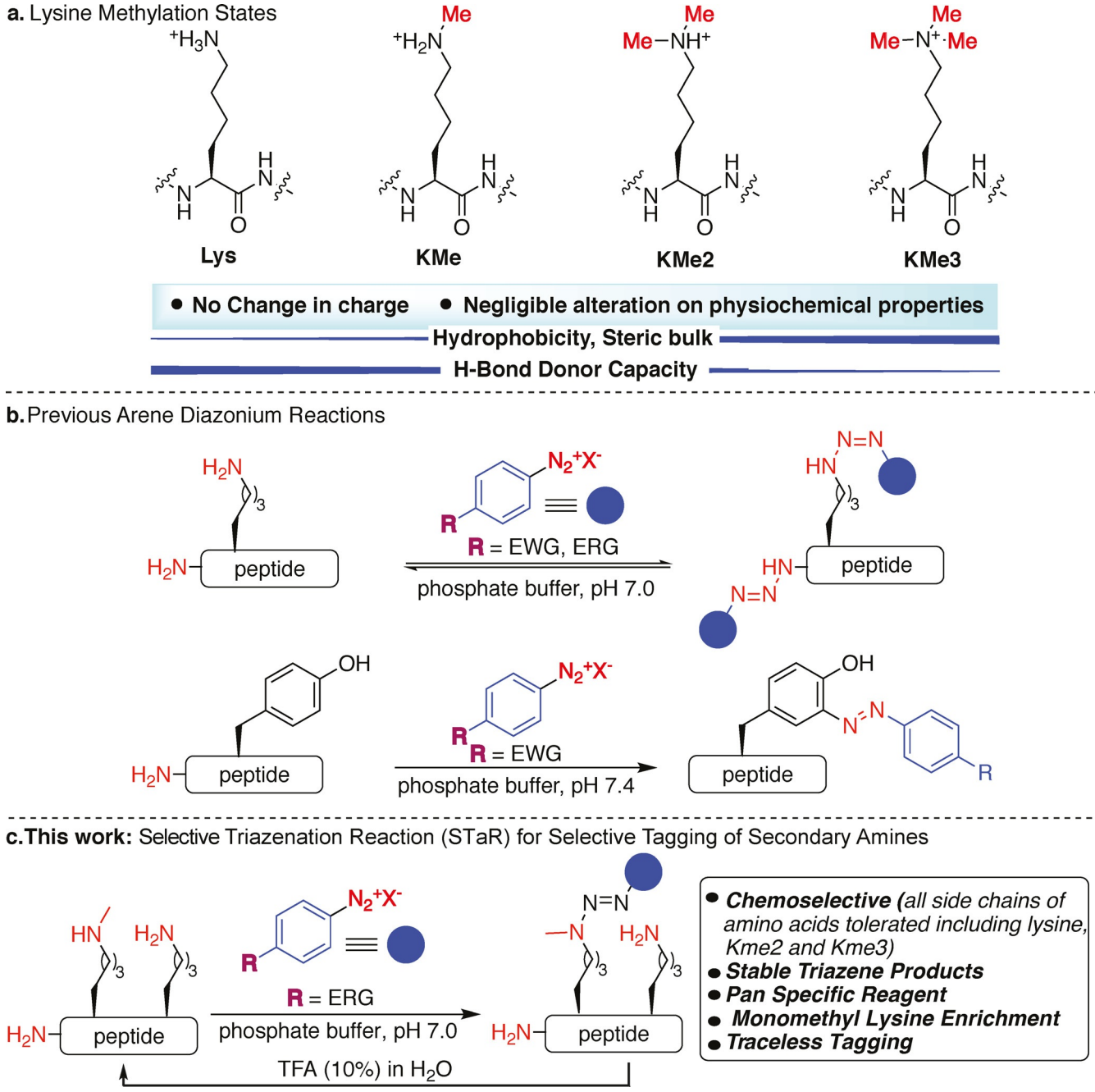

Figure 1. Selective Triazenation Reaction "STaR" a) Different methylation states of lysine and challenges with selective tagging of monomethyl lysine. b) Previous reactions with arene diazonium salts. Arene diazonium salts react with primary amines generating a reversible adduct. Selective tagging of tyrosine to form azo coupling product with electron-deficient arene diazonium salts under physiological conditions. c) One-step, chemoselective approach for labeling of secondary amines using electron-rich arene diazonium salts to give a stable triazene adduct under physiological conditions. Traceless cleavage of the triazene-coupling adduct under acidic conditions.

generate the native unchanged coupling partners in a traceless manner under mild conditions.

This strategy is well-suited for pan-specificity because of the small size of the reagents required for STaR. There are currently no known pan-specific reagents for tagging Kme. These innovative tools for detecting PTMs would augment existing detection methods and expand the chemical tool kit available for epigenetics research.

Results and Discussion

Development of Chemoselective STaR

Inspired by observation of the facile reaction of the arene diazonium ion with secondary amine to form a stable triazene,[37,38] we reasoned that STaR might serve as an attractive starting point, owing to the flexibility of introducing various functionalities on the arene diazonium ion. Arene

diazonium ions are capable of forming triazenes with primary amines, but this has never been pursued as a bioconjugation method because of its reversible nature (Figure 1b).[37,38] The azo-coupling of arene diazonium ion with tyrosine is very well known for bioconjugation, but it requires a more electrophilic arene diazonium ion with electron withdrawing groups for carrying out the reaction under physiological conditions (pH 7.4; Figure 1b).[39–42] The less electrophilic counterparts are only able to react with Tyr at a significantly elevated pH (9.0).[43,44] Moreover, it has been reported that reaction with Cys is low yielding under physiological conditions (pH 7.5), especially with electron rich diazonium ions.[37] Drawing inspiration from these observations, we rationalized that identification of less electrophilic arene diazonium ions with electron donating substituents could form the basis of a novel chemoselective reaction for secondary amines (Figure 1c).

To evaluate the reactivity of secondary amines toward less electrophilic arene diazonium ions, we started our initial investigation by carrying out a reaction between 4-methoxybenzenediazonium ion (4MDz) and monomethyl lysine amino acid. The detailed optimization studies revealed that the reaction between the monomethyl lysine amino acid (0.6 mM) and 4MDz (0.6 mM) proceeds most efficiently in the presence of Na_2CO_3 (0.6 mM) in phosphate buffer (100 mM, pH 7) at room temperature, resulting in the formation of a stable triazene-coupling product with 99% conversion in 30 min. Next, we monitored the triazene reaction between 4MDz (0.6 mM) with a monomethyl lysine peptide, AKmeF (0.6 mM), after regular intervals of time and compared it to the corresponding reaction with tyrosine (0.6 mM) under optimized conditions (phosphate buffer (100 mM), pH 7, Figure 2). The formation of the chromophoric triazene-coupling product was detected using spectrophotometry (increased absorption at 330–390 nm) and HPLC. The results showed 93% conversion to triazene-coupling adduct in just 5 min as analyzed by HPLC (Figure 2, Supplementary Figure 1). The azo-coupling product was not observed with tyrosine even after 24 h. Similar reactivity and high selectivity towards monomethyl lysine was observed with another diazonium ion, 4-carboxybenzenediazonium (4CDz), on reaction with the peptide GAKmeF under optimized conditions (Supplementary Figure 2). To characterize the triazene-coupling products with 4MDz, reactions with proline methyl ester, 2-(methylamino) ethanol and a peptide (NHMe)Ala-Phe-OMe containing secondary amines were carried out on a large scale under the reaction conditions. The resulting products were isolated and triazenation of secondary amines was confirmed by NMR (^{1}H and ^{13}C) (Supplementary Figure 3). In contrast, no product was observed with alanine, thus reconfirming literature reports that the formation of the triazene-coupling product with primary amine is reversible in nature (Supplementary Figure 4).[37,38]

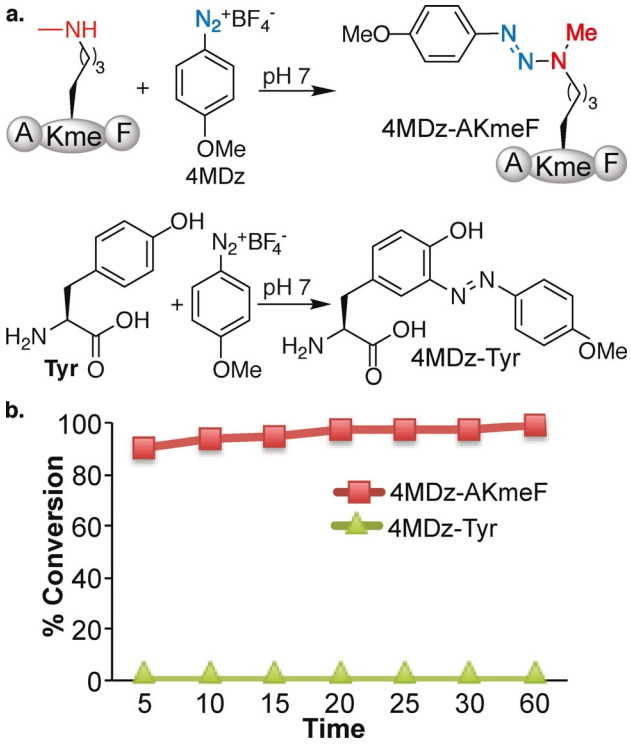

Figure 2. High reactivity of secondary amines toward electron-rich aryl diazonium ions 4MDz relative to Tyr at physiological conditions. a) The coupling reaction of 4MDz with the peptide AKmeF and Tyr. b) Observed rate of the coupling reaction of 4MDz with AKmeF and Tyr at 0.6 mM in 100 mM phosphate buffer (pH 7, 25 °C). Each time point represents an average of three independent experiments.

Chemoselectivity of STaR

To evaluate the chemoselective nature of the STaR, we carried out reaction of 4MDz with various reactive amino acids such as Ala, Asp, His, Asn, Pro, Arg, Ser, Trp, and Tyr under optimized reaction conditions. The reactions were monitored for 30 min, 2 h, and 24 h by LCMS. The data showed the formation of triazene-coupling product with proline only (Figure 3a, Supplementary Figure 5). Further, reaction of 4MDz with unmethylated lysine and various methylation states of lysine (Kme, Kme2, and Kme3) confirmed high chemoselectivity of STaR for the monomethyl lysine Kme (Figure 3b, Supplementary Figure 6). Next, we carried out STaR on peptides (GDAKF, GYARF, GWAHF, and GSANF, and DRVYIHPF) with reactive amino acids, but we did not observe any modification of peptides under the optimized conditions (Figure 3c, Supplementary Figure 7).

Pan-Specificity of STaR

Having established the optimal conditions, we sought to demonstrate the pan-specificity of STaR by carrying out reactions of 4MDz and 4CDz with various peptides of different sizes and amino-acid composition, containing monomethylated lysine at different positions. As outlined in Figure 3c, peptides bearing aromatic and aliphatic amino acids next to the monomethyl lysine were fully tolerated in this protocol by yielding the corresponding triazene-coupling products with excellent conversions (90–98%, Figure 3c, HRMS-Supplementary Figure 8). The reactions with a peptide GEPGIAGFKmeGEQGPK (collagen fragment) bearing

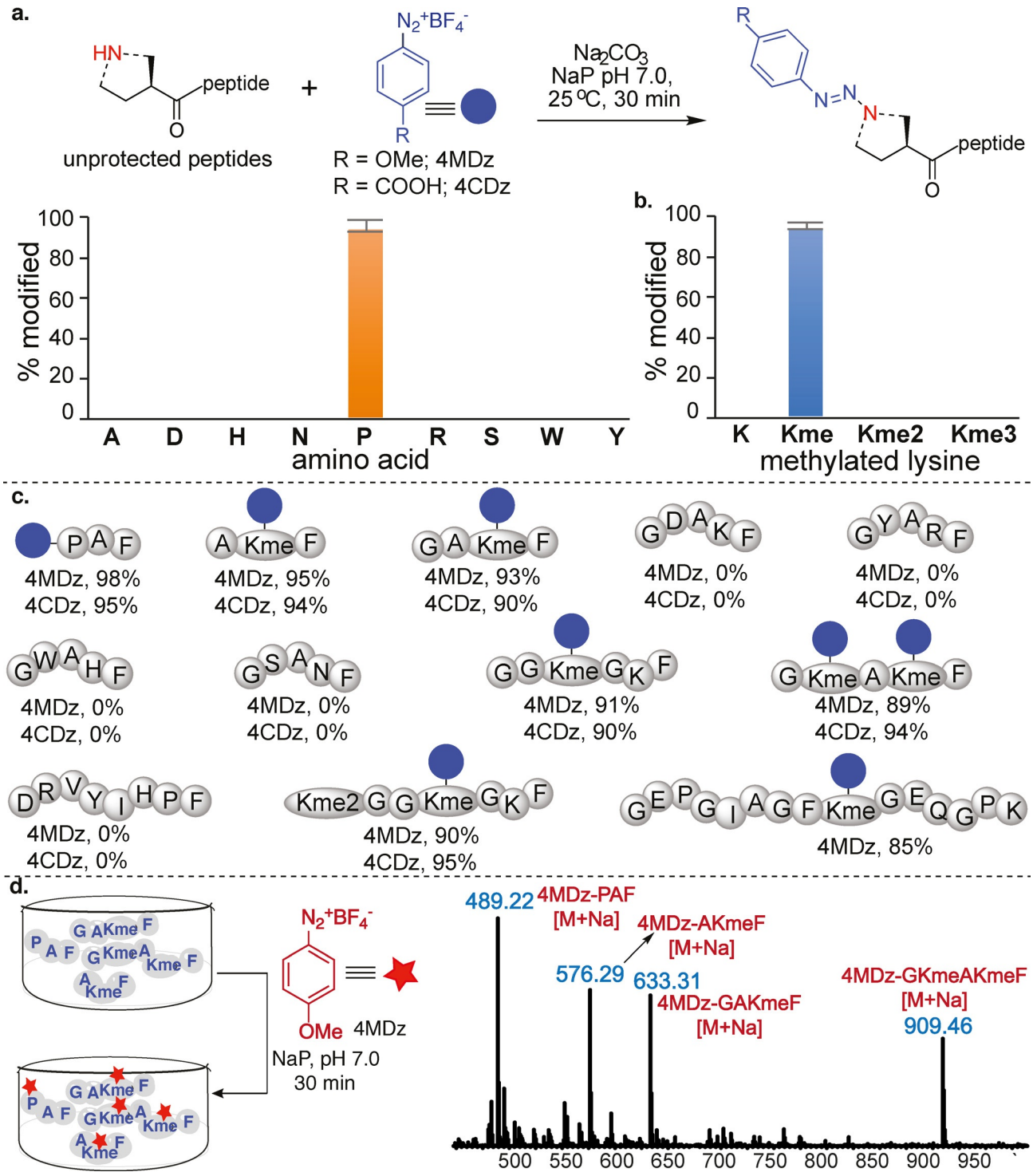

Figure 3. Secondary amine polypeptide modification using electron-rich aryl-diazoniums. a) The general reaction scheme for secondary amine triazene formation. The reaction showed high chemoselectivity of 4MDz for proline as quantified by LC/MS among a panel of reactive amino acids. b) The reaction showed high chemoselectivity of 4MDz for monomethyl lysine (Kme) among a panel of other methylated states of lysine as quantified by LC/MS. c) Chemoselective triazenation of peptides showed tolerance of amino acid side chains with 4MDz and 4CDz. d) Selective tagging of all the peptides in a complex mixture using 4MDz as analyzed by LCMS. Conditions for (a)–(c) peptide/amino acid (0.6 mM), 4MDz or 4CDz (1 equiv, 0.6 mM), Na_2CO_3 (1 equiv, 0.6 mM), phosphate buffer (NaP, 100 mM) at pH 7.0 at room temperature. The addition of Na_2CO_3 changed the pH to 8.3. For peptide GKmeAKmeF with two Kme, double equivalents of 4MDz and Na_2CO_3 were added. The samples were incubated for 30 min in (a) and (b), for 2 h in (c) and (d). Yields of products were analyzed by LCMS.

reactive amino acids, such as Glu, Gln, and Lys, did not interrupt or influence the triazenation process, and afforded a desired triazene-coupling product with Kme in good conversion (85%, Figure 3c). Interestingly, the peptide GKmeAKmeF with two monomethyl lysines at the alternate position showed the double modification due to the formation of triazene-coupled product with both monomethyl lysines (89%, Figure 3c, HRMS-Supplementary Figure 8). Together, these results confirmed the high chemoselectivity and pan-specificity of the STaR toward secondary amines. To determine the high selectivity of STaR in a complex environment, a mixture of peptides PAF, AKmeF, GAKmeF, and GKmeAKmeF was treated with 4MDz and resulting solution was analyzed by LC-MS (Figure 3d, Supplementary Figure 9). We observed complete labeling of all the peptides. These experiments demonstrate the high pan-specificity of STaR from a single peptide to a complex mixture under mild physiological conditions.

Synthesis of Arene Diazonium Affinity Tags for Monomethyl Lysine

The effective bioconjugation reaction for enrichment should have the ability to attach affinity tags, thus we generated the alkyne-functionalized aniline derived from a single step by the amidation of 4-aminobenzoic acid with propargyl amine (Figure 4a, Supplementary Figure 10). We then converted the alkyne-functionalized aniline to alkyne-functionalized diazonium ion, 4-aminobenzoic acid alkyne-diazonium ion (ABDz) in situ by treatment with sodium nitrite (Supplementary Figure 11). The STaR with ABDz showed high chemoselectivity for monomethyl lysine on reaction with peptides, such as GAKmeF, AKmeF, GGKmeGKF, GKmeAKmeF, and Kme2GGKmeGKF, under optimized conditions (89–95%, Figure 4a, Supplementary Figure 11).

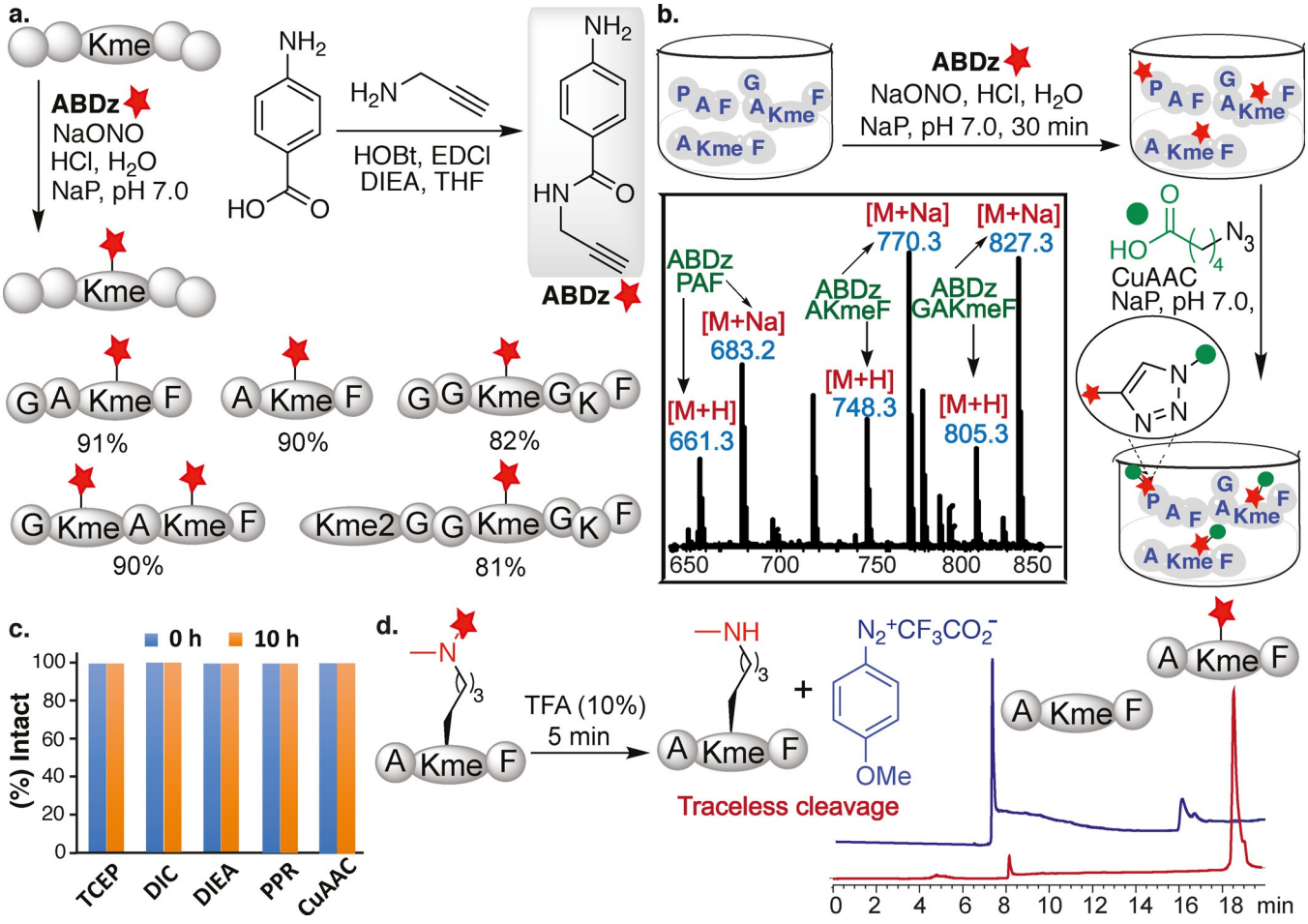

Figure 4. Chemoselective enrichment of secondary amine peptides using alkyne-functionalized arene diazonium. a) Synthetic scheme for alkyne-functionalized aniline and selective tagging of monomethyl lysine in varying peptides by in situ generation of alkyne-arene diazonium ABDz from a corresponding aniline derivative. b) Chemoselective triazenation of all the peptides in a complex mixture by in situ generated alkyne-arene diazonium ABDz followed by click chemistry with azide for further functionalization of the triazene-coupling product. c) High stability of triazene-secondary amine adduct towards a variety of bases (DIEA and PPR), coupling reagents (DIC), reducing reagent (TCEP), and click-chemistry conditions. d) Traceless cleavage of triazene-coupling product 4MDz-AKmeF under mild acidic conditions. LC-MS analysis of this reaction shows the production of the unmodified starting material, AKmeF.

Selective Tagging of Monomethyl Lysine in the Complex Mixture Using STaR

As a further demonstration of the high selectivity of STaR for monomethyl lysine, we attempted tagging multiple peptides in the same solution to test the potential of our method for enrichment in a complex mixture. The mixture of peptides PAF, AKmeF, and GAKmeF was allowed to react for 1 h under the optimized reaction conditions with alkyne-derivative of diazonium ion ABDz. This was followed by copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC)[45] with 5-azidopentanoic acid in the reaction mixture for 2 h. The reaction products were analyzed by MS and the data showed the tagging with ABDz and enrichment with 5-azidopentanoic acid for all the peptides in the reaction mixture (Figure 4b, Supplementary Figure 12).

Traceless Enrichment of Monomethyl Lysine Using STaR

We tested the chemical stability of the triazene-coupling product under different conditions. The triazene-coupling product acts as a protecting group for secondary amines and tolerates various coupling reagents, such as N,N′-diisopropylcarbodiimide (DIC), and bases, such as N,N-diisopropylethylamine (DIEA) and pyridine (PPR), which is required for Fmoc peptide synthesis (Figure 4c, Supplementary Figure 13). It is also stable to a strong protein disulfide reducing agent tris(2-carboxyethyl)phosphine (TCEP) and click chemistry (CuAAC) conditions (Figure 4c, Supplementary Figure 13). The triazene-linkage showed reasonable stability to sodium dithionate and was stable for 1 h (Supplementary Figure 14). This is in contrast to the stability of the azo-coupling product with Tyr, which degrades quickly in the presence of sodium dithionate to generate modified tyrosine.[43,44] The triazene-coupling product 4MDz-Pro-OMe was incubated in water:ACN mixture for 24 h and no decomposition of the triazene-coupling product was observed.

Even though the triazene-secondary amine linkage is stable under physiologically relevant conditions, we aimed to explore if the conjugate can be decoupled in a traceless manner. Indeed, a slight exposure of the triazene-modified peptide 4MDz-AKmeF to acidic conditions (10% TFA in H_2O) led to its rapid cleavage in 5 min to unchanged peptide AKmeF, as observed by HPLC and MS analysis (Figure 4d, Supplementary Figure 15).

It is noteworthy that the acidic cleavage generated original coupling partners in an unperturbed manner. In contrast, a handful of the coupling reactions are reversible, but the products derived from decoupling are modified versions of the original reactants.[44] The remarkable speed and selectivity of the STaR, and the ability to subsequently cleave the resulting triazene-linkage in a traceless manner to regenerate the unmodified starting materials, underscores unique advantages of this conjugation strategy.

Enrichment of Monomethyl Lysine (Kme) from Complex Mixture

Next, we established rapid, near-quantitative, and site-specific enrichment of monomethyl lysine peptides from a complex mixture of proteolytic fragments by STaR. Trypsin digested proteolytic mixture spiked with various peptides PAF, AKmeF, and GAKmeF was incubated with ABDz for 1 h, followed by incubation with azide-functionalized resins under click chemistry conditions for 2 h (Figure 5). The resin was then thoroughly washed with solvents to remove any untrapped proteolytic fragments. The enriched peptides were recovered by cleaving them from resin under acidic conditions (10% TFA in DCM) for 10 min followed by LCMS analysis (Figure 5, Supplementary Figure 16). The results showed the capturing of all the peptides containing secondary amines from the complex mixture.

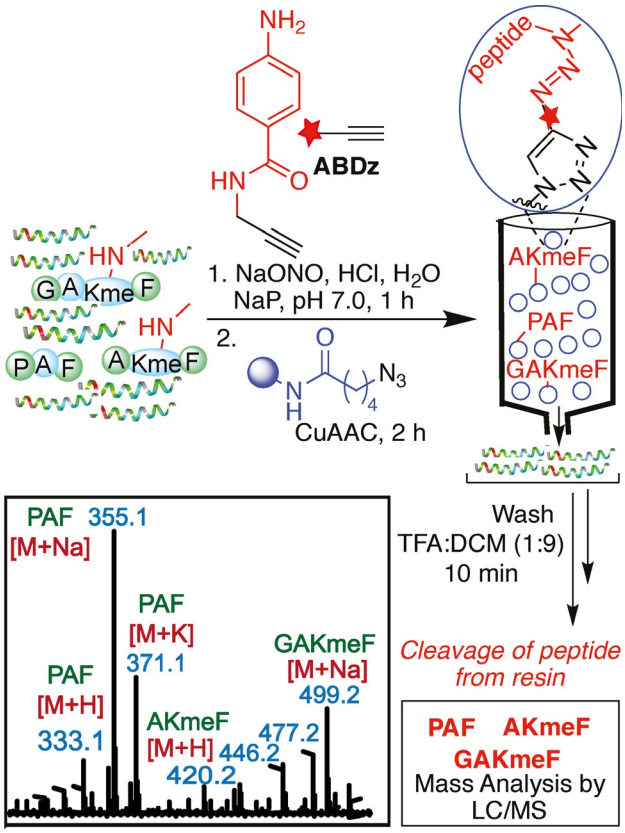

Figure 5. Site-selective enrichment of secondary amine peptides from a complex mixture. Trypsin digested proteolytic fragments spiked with secondary amine peptides were treated with alkyne-functionalized arene diazonium ion ABDz followed by trapping of triazene-coupling adducts on azide-functionalized beads using click chemistry. The trapped peptides on beads were analyzed by traceless cleavage from the beads under acidic conditions using LCMS, confirmed trapping of secondary-amine-containing peptides.

STaR for Solid-Phase Peptide Capture of Monomethyl Lysine and Release for High-Purity Samples for Proteomics

The efficient characterization of monomethyl-lysine-containing peptides from proteolytic fragments is essential, but

unfortunately their detection in the complex mixture is highly challenging. One way to selectively trap and detect the monomethyl lysine PTM with high efficiency from the complex mixture is to covalently bind monomethyl lysine peptides directly to the solid support followed by their detachment from the solid support in the traceless manner. Currently, there are no methods available in literature to achieve this goal.

We felt that this could be achieved by carrying out STaR directly on a solid support. Toward this end, arene-diazonium-ion-functionalized resin was synthesized in two steps from 5-amino-2-chloro benzylalcohol (Supplementary Figure 17).[46,47] The arene-diazonium-ion-functionalized resin was incubated with mixture of peptides AKmeF, SVF, NAF, and RAF containing primary amines and other reactive side chains for 16 h followed by the extensive washing of beads with water and various organic solvents, such as ACN, DMF, MeOH, and DCM, to remove non-covalently attached peptide fragments. The trapped peptides were released from the resin under mild acidic conditions using 10% TFA/DCM for 10 min (Supplementary Figure 18). High mass intensity of the unmodified monomethyl lysine peptide AKmeF was observed after the cleavage from the resin without any further purification. Masses of peptides without secondary amines SVF, NAF, and RAF were not observed. To determine the compatibility of this method in a complex mixture, diazonium-ion-functionalized resin was incubated with trypsin-digested protein mixture spiked with monomethylated peptide AKmeF for 16 h followed by excessive washings to remove untrapped peptides from the resin (Figure 6a).

Finally, the trapped peptides were released under mild conditions (10% TFA/DCM) and unambiguously characterized by MS to confirm the enrichment of AKmeF from a complex mixture (Figure 6a, Supplementary Figure 19). We were able to develop the STaR to enrich and analyze the monomethyl lysine peptides from a complex mixture by capturing them on a solid support and by reversing it in a traceless manner to release highly pure, unmodified peptides. Since the acidic cleavage regenerates the unmodified diazonium-ion-functionalized resin, we attempted to reuse it again to capture another set of monomethyl lysine peptide fragments from a complex mixture. To test the reusability, we first incubated the diazonium-ion-functionalized resin with peptides AKmeF and GAKmeF and Na_2CO_3 for 1 h followed by washing and detachment under mild acidic conditions (10% TFA in DCM in 10 min). The MS analysis confirmed the attachment of both AKmeF and GAKmeF peptides on the resin (Figure 6b). This is followed by the incubation of the cleaved resin with another peptide PAF to avoid any kind of false results due to the first peptide capturing. Again, MS analysis after decoupling confirmed the attachment of PAF peptide on the resin. We repeated this process a third time with a peptide AKmeF and each time we observed the enrichment of the secondary amine peptide using the same resin (Figure 6b, Supplementary Figure 20). We were able to reuse the resin thrice to enrich monomethyl lysine/secondary amine peptides without any significant loss in the reactivity of the resin. We next performed experiments to enrich secondary-amine-containing peptides from a complex mixture of proteins using a biotin-functionalized probe followed by streptavidin enrichment (Supplementary Figure 21). The trapped peptides were released in a traceless manner under acidic conditions from streptavidin beads and the reaction was analyzed by LCMS (Supplementary Figure 21). The results indicated that secondary-amine-containing peptides were enriched selectively from a complex mixture. Next, the peptide trapping was determined in a quantitative manner by incubating peptides (NHMe)AVF and GAKmeF with arene-diazonium ion resin followed by measuring the reduction in the peptide concentration in solution using anisole as standard over a 2 h period at room temperature using LCMS. The results indicated the trapping of 56% of peptides in 1 h and 87% of peptides in 2 h (Figure 6c, Supplementary Figure 22).

Conclusion

In summary, STaR provides a novel, single-step, secondary-amine-selective label, capture, and release strategy for the enrichment and identification of monomethyl lysine proteolytic fragments from a complex mixture. The STaR 1) selectively labels secondary amines/monomethyl lysine under physiological conditions, without the need for coupling reagents and metals; 2) is chemoselective for monomethyl lysine (Kme) rather than any other amino acid residues, including lysine and other methylation states of lysine (Kme2 and Kme3); 3) exhibits high reaction kinetics and efficiently tags monomethyl lysine even at low concentrations and in a complex mixture; 4) can systematically introduce multiple functional groups, including affinity tags, by rational substitution on the arene-diazonium ion, and 5) cleaves the triazene-linkage to generate unchanged starting materials in a traceless manner under mild acidic conditions. The potency of the STaR for pan-specificity is well demonstrated by the selective labeling of all the monomethyl lysine peptides in a complex mixture under physiological conditions as shown in Figures 4–5. Another attractive feature of STaR is the compatibility with existing bio-orthogonal conjugation reactions, such as azide-alkyne click reaction, thus enabling site-specific labeling of proteolytic fragments with multiple affinity tags for the enrichment of peptides from a complex mixture. Moreover, this chemistry was used for binding of monomethyl lysine peptides on a solid support in a covalent manner leading to their efficient enrichment, quantification, and purification from a complex mixture, thus providing an excellent tool for the unambiguous analysis of monomethyl lysine PTMs. Considering the simple setup of this bioconjugation reaction with easily derivatized arene-diazonium ions, we anticipate that this method will become a highly useful tool for the selective functionalization of antibodies and proteins. In general protocol for the enrichment of Kme peptides from proteolytic digested fragments, iodoacetamide could be added to block all the cysteines and also Kme peptides can be selectively released from the beads in a traceless manner under mildly acidic conditions, thus we do not anticipate any interference from reactions with cysteine and tyrosine. Thus, the STaR offers a chemical

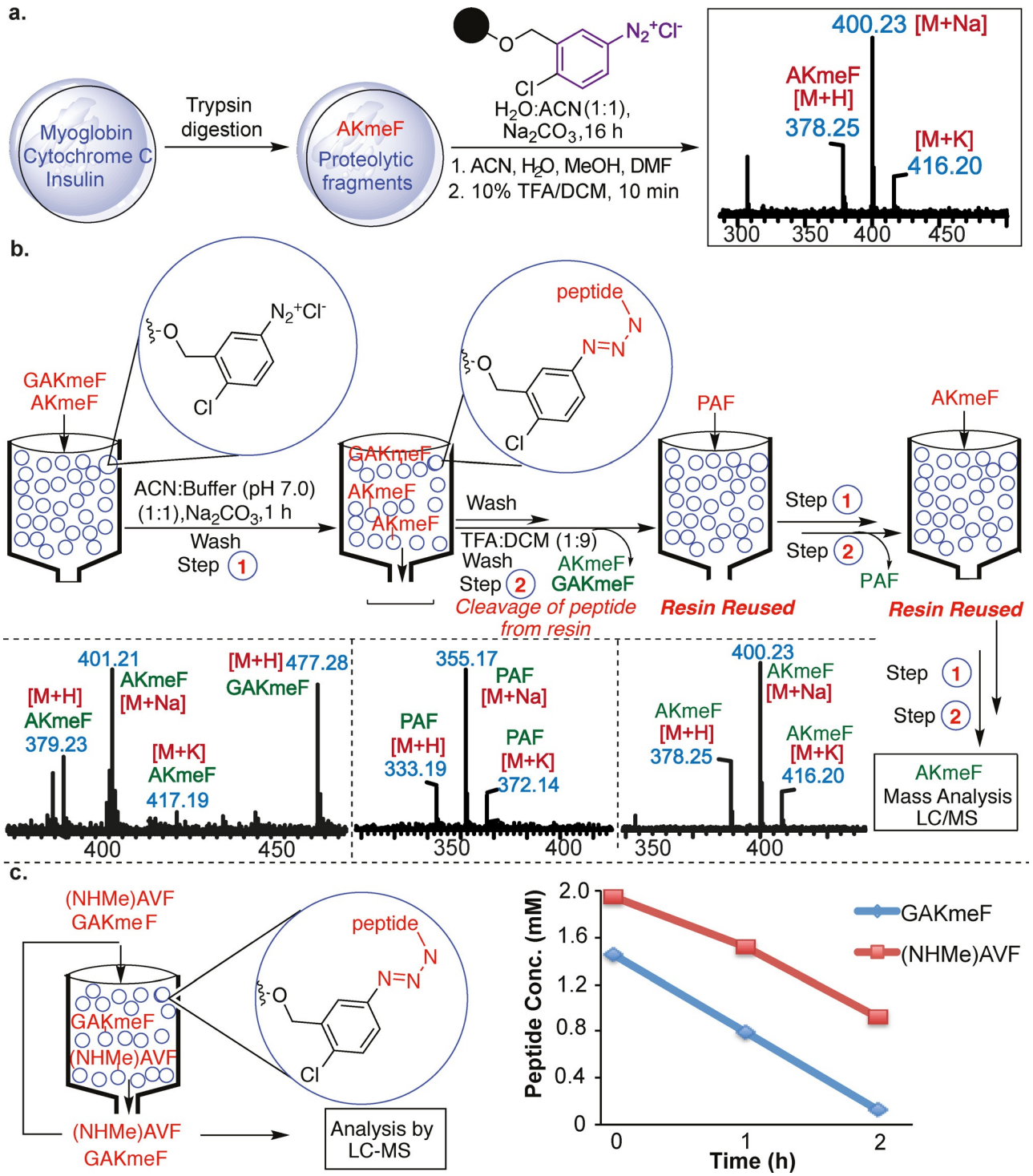

Figure 6. Selective enrichment of secondary amines from a complex mixture using an arene-diazonium-functionalized solid support. a) Selective enrichment of the monomethyl lysine peptide AKmeF from a complex mixture of proteolytic fragments using an arene-diazonium-functionalized solid support, followed by detachment under mild acidic conditions to give an unmodified peptide as analyzed by MS. b) Reusability of the arene-diazonium-functionalized resin for the selective enrichment of secondary amine peptides, such as AKmeF, GAKmeF, and PAF, in three different cycles. The results showed reusability of resin three times without any significant loss of activity. c) Quantification of the peptide trapping by the arene-diazonium-functionalized resin. Conditions for (a) and (b) peptide (1 equiv), resin (25 equiv), Na_2CO_3 (2 equiv/peptide) H_2O:ACN (1:1) at room temperature for 16 h incubation for (a) and 1 h for (b), washing of resin with ACN, water, MeOH, and DMF, and peptide detachment from resin by 10% TFA in DCM for 10 min. Analysis of the detached peptides by LCMS. For (b), Repeat the above steps three times with different peptides.

platform for identifying and studying the role of monomethyl lysine (Kme) in the whole proteome, as well as a starting point for therapeutic interventions. Since, this reaction is highly selective for secondary amines, it can be used for the bioconjugation of proteins containing proline at the N-terminus with a variety of different moieties, such as fluorophores, PEG, drugs, affinity reagents, such as alkyne and biotin, and also for the synthesis of antibody–drug conjugates. The work in these directions is currently underway in our laboratory.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: bioconjugation · chemoselectivity · monomethyl lysine · protein modifications · proteomics

[1] M. T. Bedford, S. Richard, *Mol. Cell* **2005**, *18*, 263 – 272.
[2] A. N. Lake, M. T. Bedford, *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **2007**, *618*, 91 – 101.
[3] K. S. Egorova, O. M. Olenkina, L. V. Olenina, *Biochemistry* **2010**, *5*, 535 – 548.
[4] X. Z. Yao, W. H. Shen, *Chin. Sci. Bull.* **2011**, *56*, 3493 – 3499.
[5] E. Blackwell, S. Ceman, *Mol. Reprod. Dev.* **2012**, *79*, 163 – 175.
[6] K. Kerr, H. McAneney, C. Flanagan, A. P. Maxwell, A. J. McKnight, *BMC Nephrol.* **2019**, *20*, 1 – 9.
[7] X. Zhang, H. Wen, X. Shi, *Acta Biochim. Biophys. Sin.* **2012**, *44*, 14 – 27.
[8] Z. Wu, J. Connolly, K. K. Biggar, *FEBS J.* **2017**, *284*, 2732 – 2744.
[9] J. Huang, S. L. Berger, *Curr. Opin. Gen. Dev.* **2008**, *18*, 152 – 158.
[10] J. Huang, L. Perez-Burgos, B. J. Placek, R. Sengupta, M. Richter, J. A. Dorsey, S. Kubicek, S. Opravil, T. Jenuwein, S. L. Berger, *Nature* **2006**, *444*, 629 – 632.
[11] K. K. Biggar, S. S. Li, *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 5 – 17.
[12] R. Hamamoto, V. Saloura, Y. Nakamura, *Nat. Rev. Cancer* **2015**, *15*, 110 – 124.
[13] R. Hamamoto, Y. Furukawa, M. Morita, Y. Limura, F. P. Silva, M. Li, R. Yagyu, Y. Nakamura, *Nat. Cell Biol.* **2004**, *6*, 731 – 740.
[14] Y. Kotake, R. Cao, P. Viatour, J. Sage, Y. Zhang, Y. Xiong, *Genes Dev.* **2007**, *21*, 49 – 54.
[15] R. Hamamoto, F. P. Silva, M. Tsuge, T. Nishidate, T. Katagiri, Y. Nakamura, Y. Furukawa, *Cancer Sci.* **2006**, *97*, 113 – 118.
[16] A. D. Ferguson, N. A. Larsen, T. Howard, H. Pollard, I. Green, C. Grande, T. Cheung, R. Garcia-Arenas, S. Cowen, J. Wu, R. Godin, H. Chen, N. Keen, *Structure* **2011**, *19*, 1262 – 1273.
[17] L. X. Li, L. X. Fan, J. X. Zhou, J. J. Grantham, J. P. Calvet, J. Sage, X. Li, *J. Clin. Invest.* **2017**, *127*, 2751 – 2764.
[18] F. Casciello, K. Windloch, F. Gannon, J. S. Lee, *Front Immunol.* **2015**, *6*, 487.
[19] E. L. Greer, Y. Shi, *Nat. Rev. Genet.* **2012**, *13*, 343 – 357.
[20] H. Zhou, M. Ye, J. Dong, G. Han, X. Jiang, R. Wu, H. Zou, *J. Proteome Res.* **2008**, *7*, 3957 – 3967.
[21] Y. Chen, W. Zhao, J. S. Yang, Z. Cheng, H. Luo, Z. Lu, M. Tan, W. Gu, Y. Zhao, *Mol. Cell. Proteomics* **2012**, *11*, 1048 – 1062.
[22] P. Hägglund, J. Bunkenborg, F. Elortza, O. N. Jensen, P. Roepstorff, *J. Proteome Res.* **2004**, *3*, 556 – 566.
[23] B. Polevoda, F. Sherman, *Mol. Microbiol.* **2007**, *65*, 590 – 606.
[24] M. Bremang, A. Cuomo, A. M. Agresta, M. Stugiewicz, V. Spadotto, T. Bonaldi, *Mol. BioSyst.* **2013**, *9*, 2231 – 2247.
[25] Q. Wang, K. Wang, Y. Mingliang, *Analyst* **2017**, *142*, 3536 – 3548.
[26] V. Geoghegan, A. Guo, D. Trudgian, B. Thomas, O. Acuto, *Nat. Commun.* **2015**, *6*, 6758.
[27] A. Guo, H. Gu, J. Zhou, D. Mulhern, Y. Wang, K. A. Lee, V. Yang, M. Aguiar, J. Kornhauser, X. Jia, J. Ren, S. A. Beausoleil, J. C. Silva, V. Vemulapalli, M. T. Bedford, M. J. Comb, *Mol. Cell. Proteomics* **2014**, *13*, 372 – 387.
[28] K. E. Moore, S. M. Carlson, N. D. Camp, P. Cheung, R. G. James, K. F. Chua, A. Wolf-Yadlin, O. Gozani, *Mol. Cell* **2013**, *50*, 444 – 456.
[29] S. M. Carlson, K. E. Moore, E. M. Green, G. M. Martin, O. Gozani, *Nat. Protoc.* **2014**, *9*, 37 – 50.
[30] I. Bock, A. Dhayalan, S. Kudithipudi, O. Brandt, P. Rathert, A. Jeltsch, *Epigenetics* **2011**, *6*, 256 – 263.
[31] S. Nishikori, T. Hattori, S. M. Fuchs, N. Yasui, J. Wojcik, A. Koide, B. D. Strahl, S. Koide, *J. Mol. Biol.* **2012**, *424*, 391 – 399.
[32] M. Busby, C. Xue, C. Li, Y. Farjoun, E. Gienger, I. Yofe, A. Gladden, C. B. Epstein, E. M. Cornett, S. B. Rothbart, C. Nusbaum, A. Goren, *Epigenet. Chromatin* **2016**, *9*, 49.
[33] L. A. Ingerman, M. E. Cuellar, M. L. Waters, *Chem. Commun.* **2010**, *46*, 1839 – 1841.
[34] K. I. Albanese, M. W. Krone, C. J. Petell, M. M. Parker, B. D. Strahl, E. M. Brustad, M. L. Waters, *ACS Chem. Biol.* **2020**, *15*, 103 – 111.
[35] N. L. Young, P. A. DiMaggio, M. D. Plazas-Mayorca, R. C. Baliban, C. A. Floudas, B. A. Garcia, *Mol. Cell. Proteomics* **2009**, *8*, 2266 – 2284.
[36] Y. E. Sim, O. Nwajiobi, S. Mahesh, R. D. Cohen, M. Y. Reibarkh, M. Raj, *Chem. Sci.* **2020**, *11*, 53 – 61.
[37] S. Sengupta, S. Chandrasekaran, *Org. Biomol. Chem.* **2019**, *17*, 8308 – 8329.
[38] A. Sonousi, D. Crich, *Org. Lett.* **2015**, *17*, 4006 – 4009.
[39] T. L. Schlick, Z. Ding, E. W. Kovacs, M. B. Francis, *J. Am. Chem. Soc.* **2005**, *127*, 3718 – 3723.
[40] M. A. Bruckman, G. Kaur, L. A. Lee, F. Xie, J. Sepulveda, R. Breitenkamp, X. Zhang, M. Joralemon, T. P. Russell, T. Emrick, Q. Wang, *ChemBioChem* **2008**, *9*, 519 – 523.
[41] J. Gavrilyuk, H. Ban, M. Nagano, W. Hakamata, C. F. Barbas, *Bioconjugate Chem.* **2012**, *23*, 2321 – 2328.
[42] S. Chen, M. L. Tsao, *Bioconjugate Chem.* **2013**, *24*, 1645 – 1649.
[43] J. M. Hooker, E. W. Kovacs, M. B. Francis, *J. Am. Chem. Soc.* **2004**, *126*, 3718 – 3719.
[44] P. S. Addy, S. B. Erickson, J. S. Italia, A. Chatterjee, *J. Am. Chem. Soc.* **2017**, *139*, 11670 – 11673.
[45] B. M. Cornali, F. W. Kimani, J. C. Jewett, *Org. Lett.* **2016**, *18*, 4948 – 4950.
[46] S. Bräse, S. Dahmen, F. Lauterwasser, N. E. Leadbeater, E. L. Sharp, *Bioorg. Med. Chem. Lett.* **2002**, *12*,1845 – 1848.
[47] R. Lazny, A. Nodzewska, P. Klosowski, *Tetrahedron* **2004**, *60*, 121 – 130.

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