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 **Very Important Paper**

Covalent Labeling of Matrix Metalloproteases with Affinity-Based Probes Containing Tuned Reactive N-Acyl-N-Alkyl Sulfonamide Cleavable Linkers

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Original covalent probes with an N-acyl-N-alkyl sulfonamide cleavable linker were developed to target a broad set of human Matrix Metalloproteases (MMPs). The electrophilicity of this cleavable linker was modulated to improve the selectivity of the probes as well as reduce their unspecific reactivity in complex biological matrices. We first demonstrated that targeting the S₃ subsite of MMPs enables access to broad-spectrum affinity-based probes that exclusively react with the active version of these proteases. The probes were further assessed in proteomes

of varying complexity, where human MMP-13 was artificially introduced at known concentration and the resulting labeled MMP was imaged by in-gel fluorescence imaging. We showed that the less reactive probe was still able to covalently modify MMP-13 while exhibiting reduced off-target unspecific reactivity. This study clearly demonstrated the importance of finely controlling the reactivity of the NASA warhead to improve the selectivity of covalent probes in complex biological systems.

Introduction

Covalent modification of native proteins with small-molecule probes is a powerful approach that finds numerous applications in both basic and applied research.^[1,2] For instance, fluorescent labeling of proteins in their biological environments enables their direct visualization as well as the study of their molecular dynamics in real time.^[3-5] On the other hand, covalent inhibitors that provide sustainable enzyme inhibition have recently seen a strong resurgence in various pathological contexts and are revolutionizing the treatment paradigms of several cancers.^[6,7] Over the past two decades, activity-based protein profiling,^[8,9] which utilizes activity-based probes (ABPs) to covalently modify the active sites of enzymes, has also gradually become one of the major techniques in proteomics for documenting the functional states of enzymes in various


biological matrices, including in vivo. This approach has been extensively explored within the large family of proteases,^[10-12] to image them^[13-15] or to validate them as potential therapeutic targets in human non-communicable diseases,^[16] as well as during parasitic^[17] or bacterial infections.^[18]


Most activity-based probes (ABPs) incorporate three key structural elements in their design: i) a reactive electrophilic group (warhead), ii) a recognition motif to guide the proximity-driven reaction between a nucleophile within the enzyme's catalytic site and the electrophilic warhead, and iii) an analytical tag (e.g., a fluorescent reporter or biotin) for subsequent visualization and identification of the covalently labeled enzyme. Specifically for proteases, two types of active site-directed probes have been developed: i) those targeting a nucleophile directly involved in the catalytic mechanism, namely a cysteine or serine residue found in the catalytic cleft of cysteine and serine proteases respectively,^[10-12] and ii) those reacting with non-canonical nucleophiles located near the protease active site. This second category primarily includes reactive probes targeting metalloproteases, which lack a canonical nucleophile involved in catalysis.^[19] In this case, the affinity-based probes (AfBPs) typically feature i) a recognition motif comprising a pseudopeptide core with a zinc-binding group (ZBG) to chelate the catalytic zinc ion, and ii) a photoactivable group as the reactive warhead, which upon photoactivation becomes reactive enough to form a covalent bond with an amino acid in close proximity.^[19] Although these AfBPs are valuable for profiling active metalloproteases in different biological matrices, their use is mainly restricted to ex vivo applications due to the irradiation conditions, which are not compatible with in vivo constraints.

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To partially circumvent this limitation, our group has recently developed a new generation of AfBPs that can react with matrix metalloproteases (MMPs) without any external trigger.^[20,21] These probes are based on a phosphinic pseudo-peptide scaffold to which cleavable linkers as leaving groups have been attached. Specifically, an acyl imidazole or an N-acyl-N-alkyl sulfonamide cleavable linker reacts with lysine residues on flexible loops within the S_3' subsite of MMPs.^[20] This proximity-driven reaction results in the attachment of an analytical tag to MMPs and the concomitant release of the recognition motif. Although these AfBPs are capable of modifying MMPs in complex proteomes, they exhibit some limitations, with only a few MMPs efficiently labeled and some off-target reactivity toward abundant proteins such as serum albumin present at high concentrations in various biological matrices.

Building on our previous observations, the present study aims to re-explore the MMP-directed labeling strategy recently developed in our laboratory along two dimensions: i) expanding its application to a broader set of MMP members, and ii) reducing its unspecific reactivity in complex biological matrices. To this end, we have designed AfBPs that i) target a subsite of MMPs where canonical nucleophiles are present, and ii) contain two NASA-derived types of cleavable linker with a significantly distinct reactivity. The developed probes were capable of transferring either a fluorescent reporter or a clickable handle for subsequent derivatization. They were assessed on both isolated MMPs and human MMP-13 introduced at known concentrations into proteomes of varying

complexity. Their labeling capacity and selectivity were examined using in-gel fluorescence imaging.

Results and Discussion

Design and Synthesis of the AfBPs: By analyzing the 3D structures and sequence alignments of MMP catalytic domains, we identified several lysine residues within their S_3 subsite that could potentially be targeted by a reactive cleavable linker (Figure 1A). Additionally, active MMPs consistently display an N-terminal end within their S_3 region (Figure 1A), which provides an additional nucleophilic amino function susceptible to react through a proximity-driven reaction. Compared to the MMP S_3' subsite, which harbors a restricted number of lysine residues (Figure S1), the S_3 subsite thus appeared optimal for targeting a broad set of MMPs. Based on this observation, we designed AfBPs with a phosphinic pseudo-peptide core harboring a phosphoryl group as ZBG and capable of projecting a cleavable linker within the MMPs' S_3 subsite (Figure 1B). Noteworthy, the phosphinic pseudo-peptide scaffold was inspired by the structure of RXP500.1, a potent broad-spectrum MMP inhibitor.^[20] For the cleavable linker, we selected the N-acyl-N-alkyl sulfonamide (NASA) motif due to its two major features: i) it efficiently and preferentially reacts with lysine residues^[20,22,23] and ii) its electrophilicity can be tuned by altering the electron-withdrawing properties of the N-alkyl group (R group, Figure 1B).^[22,24,25] Consequently, four AfBPs were developed: two

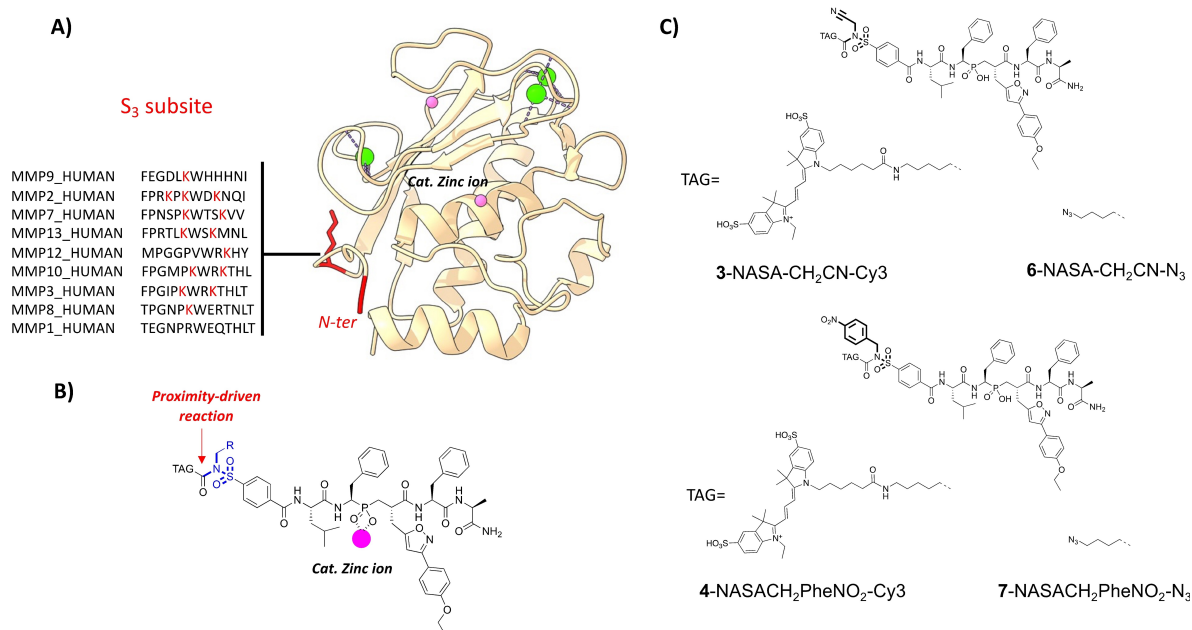


Figure 1. Design of AfBPs targeting the S_3 subsites of MMPs. A) Crystal structure of human rhMMP-12 (PDB code: 4GQL) showing the S_3 subsite where targetable Lysine residues as well as a N-terminal end (N-ter) can be found. Key: catalytic domain of rhMMP-12 (cartoon representation), zinc ion (magenta balls); calcium ion (green balls); Lysine residues in red stick and highlighted in red within the sequences of MMPs. MMPs have been aligned using uniprot (<https://www.uniprot.org>). B) General structure of AfBPs with a phosphinic pseudo peptide core projecting a N-acyl-N-alkyl sulfonamide (NASA) cleavable linker within the S_3 subsite of MMPs. C) Chemical structure of probes 3 and 6 where the NASA motif is alkylated with a cyano moiety, and that of probes 4 and 7 where the NASA motif is alkylated with a 4-nitrophenyl moiety. Probe 3 and 4 are able to transfer a Cyanine 3 reporter (Cy3) while probe 6 and 7 transfer an azido tag for subsequent derivatization.

with a sulfonamide motif alkylated with a cyano moiety (probes 3 and 6, Figure 1C) and two others with a sulfonamide motif alkylated with a 4-nitrophenyl moiety (probes 4 and 7, Figure 1C). Probes 3 and 4 can react with MMPs to transfer a fluorescent reporter (Cyanine 3, Cy@3) for their subsequent in-gel visualization. Probes 6 and 7 can transfer a small azido tag for subsequent derivatization through a click reaction.

The synthesis and characterization of the four probes are detailed in the supporting information (Schemes S1–S3, and Supplementary Methods). Briefly, the phosphinic pseudo-peptide core was first constructed on rink amide resin following a Fmoc strategy (Scheme S2), from a phosphinic building block 571 first synthesized in solution (Scheme S1). After cleavage from the support and purification via RP-HPLC, the amino intermediate 1 was further derivatized in solution with Cy@3 Mono NHS Ester. The resulting Cy@3 derivative 2 was then alkylated with either iodoacetonitrile or 4-nitrobenzyl bromide to yield probes 3 and 4, respectively, after RP-HPLC purification. The synthesis of probes 6 and 7 followed a similar synthetic pathway, with the azido moiety incorporated on the solid support prior to cleavage (Scheme S3). After cleavage and RP-HPLC purification, the azido intermediate 5 was alkylated with either iodoacetonitrile or 4-nitrobenzyl bromide to produce probes 6 and 7, respectively.

Binding Properties of AfBPs 3, 4, 6, and 7: The four probes were evaluated on a set of nine recombinant active human MMPs (hMMP-1, 2, 3, 7, 8, 9, 10, 12, and 13) through an inhibition assay with a fluorogenic substrate, as previously reported.^[20] Although potentially reactive, the four probes were regarded as simple competitive inhibitors, and an apparent IC₅₀ was determined for each. As depicted in Table 1, probe 3 exhibited significant potency towards hMMP-2, 3, 8, 9, 10, 12, and 13, with binding affinities ranging from nanomolar (hMMP-2, 3, 8, 10) to sub-nanomolar concentrations (hMMP-9, 12, and 13). Notably, similar to RXP500.1, probe 3 showed very low potency towards hMMP-1 and 7. Replacing the cyano group in probe 3 with a 4-nitrophenyl group resulted in probe 4, which exhibited reduced potency towards all tested MMPs. However, such reduction in potency was relatively modest, ranging from a factor of 2 (hMMP-9) to 10 (hMMP-10). In the cyano series, the nature of the tag had a minor impact on the binding properties of the probes, with

probe 3 featuring a Cy3 tag displaying a binding profile very similar to that of probe 6 with an azido tag. A significant and different impact of the tag on the binding profile was observed when comparing probe 4 to probe 7. This latter probe is relatively hydrophobic and its solubility in aqueous buffer is potentially not optimal, which could partly explain such behavior. Overall, the four probes demonstrated good to very good potency towards hMMP-2, 8, 9, 10, 12, and 13.

Labeling Experiments on hMMPs: The labeling capabilities of probes 3 and 4 were initially assessed on nine human recombinant MMPs (hMMP-1, 2, 3, 7, 8, 9, 10, 12, and 13). Each probe (1 μM) was incubated with a single hMMP at 40 nM. After two hours of incubation at 37 °C, the mixtures were quenched with Laemmli buffer, resolved by SDS-PAGE, and the Cy3-labeled hMMPs visualized by in-gel fluorescence imaging. As shown in Figure 2, all tested MMPs were labeled by probe 3, albeit with varying efficacy; hMMP-1 and 7 were minimally labeled, while hMMP-2, 3, 8, 9, 10, 12, and 13 were clearly tagged with the Cy3 reporter. Interestingly, two fluorescent bands were observed for labeled hMMP-3, 10, and 13. In these cases, regardless of the specific MMP, the upper band corresponds to a form containing both the catalytic and hemopexin domains,^[19] while the lower molecular weight band represents a form in which part of the hemopexin domain has been removed. These two active forms targetable by reactive AfBPs are generated during the activation process of inactive MMPs (pro form) through an autocatalytic process. Such an autolysis is particularly pronounced in the case of hMMP-12, where the activation of the pro-form (52 kDa) consistently leads to active forms in which the hemopexin domain is proteolytically removed.^[26–28] This explains why only labeled forms with molecular weights between 20 and 25 kDa were observed for this protease.

Probe 4 also reacted with hMMP-2, 3, 8, 9, 10, 12, and 13, but with a significantly lesser efficacy compared to probe 3. This observation aligns with a prior study which reported that a cyano probe targeting FKBP12 protein exhibited significantly higher reactivity than a 4-nitrophenyl one.^[22] The observed difference in reactivity between cyano and nitrophenyl probes is likely due to differences in electron-withdrawing properties and steric hindrance of the N-alkyl group, which affect the electrophilicity of the NASA linker.^[22,24,25]

Table 1. IC₅₀ (nM) of probes 3, 4, 6 and 7 toward a panel of nine MMPs. The inhibitory assays were carried out in 50 mM TRIS buffer, containing 10 mM CaCl₂, 50 μM ZnCl₂, and 0.01 % Brij 35, pH = 7.4. Continuous kinetic assays were performed by recording the fluorescence increase induced by the cleavage of fluorogenic substrate. The percentage of inhibition was determined in triplicate, at four inhibitor concentrations. ND: not determined.

rhMMP	IC ₅₀ (nM)								
	hMMP-1	hMMP-2	hMMP-3	hMMP-7	hMMP-8	hMMP-9	hMMP-10	hMMP-12	hMMP-13
500.1	>10μM	0.18±0,01	143±22	>10μM	1.6±0,1	0.060±0.01	17.8±2,5	0.21±0,02	1.07±0,08
3-NASA-CH ₂ CN-Cy3	>10μM	2.0±0,1	22±8	>10μM	2.06±0.05	0.37±0,02	2.9±0,3	0.04±0.01	0.59±0.04
4-NASA-CH ₂ PheNO ₂ -Cy3	ND	10.2±0.6	135±28	ND	6.5±0.2	0.85±0.04	22±2	0.16±0.01	1.8±0.2
6-NASA-CH ₂ CN-N ₃	>10μM	2.8±0.4	28±3	4.5μM	3.8±0.2	0.52±0.03	2.9±0.5	0.11±0.01	0.64±0.02
7-NASA-CH ₂ PheNO ₂ -N ₃	ND	96±8	1500±200	ND	82±8	8.6±1.3	103±13	3.6±0.3	19.6±1.5

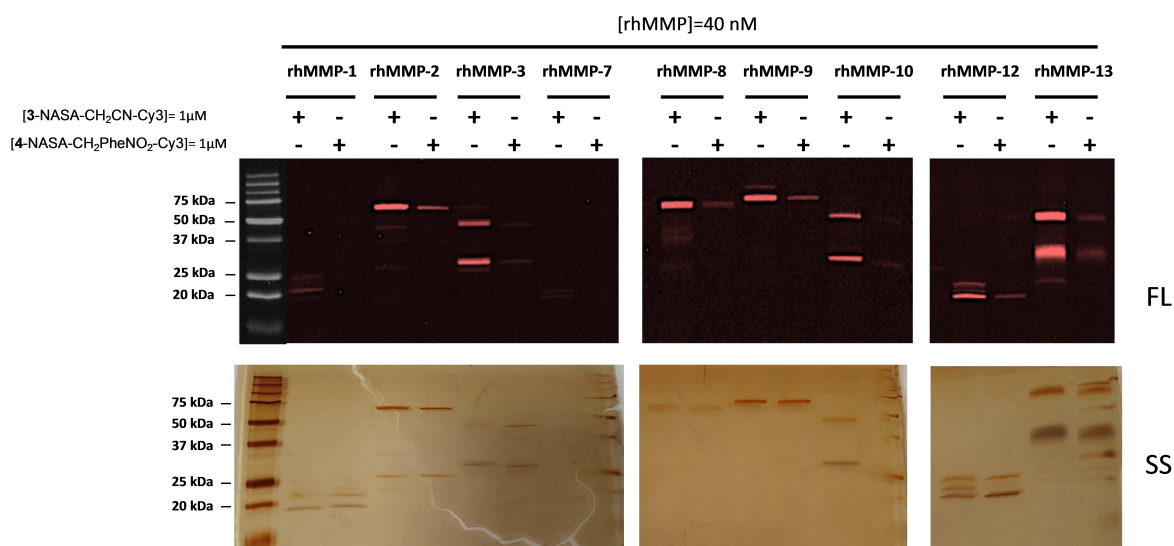


Figure 2. Labeling of human recombinant MMP with probes 3 and 4. Recombinant hMMP-1, 2, 3, 7, 8, 9, 10, 12 and 13 (40 nM) and probes 3 or 4, (1 μM) were incubated for 2 hours at 37 °C in labeling buffer (pH = 7.4, 50 mM TRIS, 10 mM CaCl₂, 50 μM ZnCl₂, containing 0.01 % Brij 35). Each sample was diluted with 4X Laemmli buffer without reductants, heated for 5 min at 95 °C before, resolved by 15 % SDS-PAGE and in-gel fluorescence imaged (FL). The presence of protein was confirmed by silver staining (SS).

Concurrently, we confirmed that probes 3 and 4 reacted only with the active form of hMMPs. To this end, we incubated pro-hMMP-2 and pro-hMMP-9 (40 nM), both featuring a propeptide domain that blocks the access to the catalytic cleft, with the probes at 1 μM for two hours at 37 °C. We demonstrated that only the active forms were labeled (Figure S2A), and in the case of pro-hMMP-2 sample the observed labeled band unambiguously corresponded to a minor fraction of in situ activated hMMP-2 in mixture with the pro form. This conclusively verified that both probes functioned as AfBPs.

We also evaluated the reactivity of probes 6 and 7 using a two-step labeling process: i) incubation of the probe (1 μM) with hMMP (40 nM) for two hours at 37 °C, followed by ii) a strain-promoted 1,3-dipolar cycloaddition reaction with dibenzocyclooctyne (DBCO)-Cy@3 at 10 μM conducted at 4 °C overnight. This procedure was applied to hMMP-2, 9 (Figure S2A), and hMMP-13 (Figure S3). Probes 6 and 7 demonstrated labeling efficacies comparable to those of probes 3 and 4, respectively, with the cyano probe 6 showing significantly higher reactivity than probe 7 with a 4-nitrophenyl motif. Both azido probes were also inactive towards pro-MMPs (Figure S2A). We further explored the click reaction with DBCO-Cy3 at both 10 μM and 1 μM with no pre-incubation of MMPs with azido probes. Quite surprisingly, we observed that hMMP-9, along with its pro form, was faintly labeled by DBCO-Cy3 (Figure S2B). Despite being unexpected, this observation aligns with previous findings on the side reactivity of cyclooctyne derivatives, which can undergo undesired conjugations with lysine and cysteine side-chains exposed onto protein surfaces.^[29]

Covalently Modified Residues within the hMMP-13 Active Site: hMMP-13 (40 nM), labeled by probe 3 (1 μM), was subjected to a standard proteomic analysis to identify the residues covalently modified within its catalytic cleft. Follow-

ing thermal denaturation combined with precipitation in cold acetone, Cy@3-labeled hMMP-13 was digested by trypsin, and the resulting digest was analyzed by MALDI TOF MS. Compared to the unlabeled hMMP-13, a single additional peak at 1520.69 Da was unambiguously detected (Figure S3A). MALDI-TOF MS/MS sequencing of this peak confirmed that it corresponded to a peptide fragment present at the N-terminal end of hMMP-13 catalytic domain (YNVFPR sequence, Figure S3B), with the N-terminal residue (Tyr104) covalently modified at its alpha amino group (Figure S3C). This labeling is consistent with a covalent modification within the S₃ subsite of hMMP-13 (Figure S3D), aligning with both the design of probe and its expected binding mode, as well as the reactivity of the NASA linker, which preferentially targets amino functions. Notably, the N-terminal amino function of YNVFPR sequence is only exposed when MMP-13 is in its active form, with its pro domain proteolytically removed (Figure S3B). This indicates that probe 3, and potentially analogous probes 4, 6, and 7, are true activity-based probes for hMMP-13.

Labeling of hMMP-13 in Complex Proteomes: We examined the reactivity of probes 3 and 4 in complex proteomes at a concentration of 1 mg/mL. The three proteomes tested were human serum albumin (hSA), mouse tumor extract, and mouse liver extracts, where we had previously observed non-specific reactivity of AfBPs with a NASA-cleavable linker.^[20] These proteomes, representing varying levels of protein complexity, were used to evaluate the probes' ability to selectively label active MMP while minimizing non-specific labeling. Active form of hMMP-13 was introduced at a known concentration (40 nM), accounting for 0.2% of the total protein content. We specifically chose hMMP-13 because it displays two distinct fluorescent bands when labeled, allowing for its unambiguous visualization using in-gel fluorescence imaging. Initially, we demonstrated that hMMP-13 could be efficiently labeled by

probes **3** and **4** used at 100 nM (Figure S4), a suitable probe concentration to minimize the nonspecific labeling.

After incubating for 2 hours at 37 °C, probe **3** (100 nM) labeled hMMP-13 (40 nM) within the three proteomes (Figure 3A, left panel). However, this probe also displayed unspecific labeling, not only with human serum albumin (hSA) but also with proteins present in tumor and liver extracts (Figure 3A, unspecific fluorescent bands are marked with white stars). In these same complex biological systems, probe **4** exhibited significantly less unspecific reactivity than probe **3** (Figure 3A, right panel), while maintaining a good labeling capacity toward hMMP-13. This result aligns with the reduced reactivity of probe **4** compared to probe **3**, clearly demonstrating that decreasing the electrophilicity of the NASA motif enhances the probe's selectivity. Notably, the off-target proteins labeled by probe **3**, and to a lesser extent by probe **4**, were not the most abundant proteins, particularly in the liver extract (Figure 3B).

We also assessed the labeling capacity of probes **6** and **7** (100 nM) in liver extract (1 mg/mL), into which hMMP-13 was artificially introduced (40 nM). This was achieved through a three-step process: (i) a labeling step at 37 °C for 2 hours; (ii) a blocking reaction with iodoacetamide (1 mM) for 30 minutes at room temperature to mitigate any potential thiol-yne addition,^[30] and (iii) a strain-promoted azide-alkyne cycloaddition with DBCO–Cy3 (1 μM) for 12 hours at 4 °C. Under these conditions, the less reactive probe **7** displayed almost

comparable reactivity to that of probe **6** (Figure 3C), strongly suggesting that some of the nonspecific labeling observed with probes **3** and **4** might be attributable to the fluorescent tag Cy3 itself. This observation supports the notion that the fluorescent reporter partly contributes to unspecific binding affinity of small-molecule probes to abundant proteins, as previously reported with phosphinic pseudo-peptides bearing a fluorescent reporter.^[31] Despite a pretreatment with iodoacetamide, two additional fluorescent bands were detected (Figure 3C, fluorescent bands marked with a blue arrow), likely corresponding to proteins labeled through a thio-yne side reaction.^[30]

MMP-Directed probes with Broad-Spectrum Profile and Improved Selectivity: In the present study, we have developed four reactive probes with a phosphinic pseudo-peptides scaffold, which enables the projection a NASA-cleavable linker within the S₃ subsite of MMP. Targeting this subsite with a NASA linker results in the labeling of all the MMPs tested, clearly validating our design for accessing broad-spectrum reactive probes. When the electrophilicity of the NASA motif is decreased by modifying the electron-withdrawing properties of the N-alkyl group (cyano vs. 4-nitrophenyl, respectively), the labeling capacity of the probes (**3** and **4**, respectively) is significantly impacted. This is likely due to a difference in the intrinsic reactivity of the NASA motif, as both probes exhibit very comparable binding properties toward the MMPs. We also demonstrated that the four probes react only with the

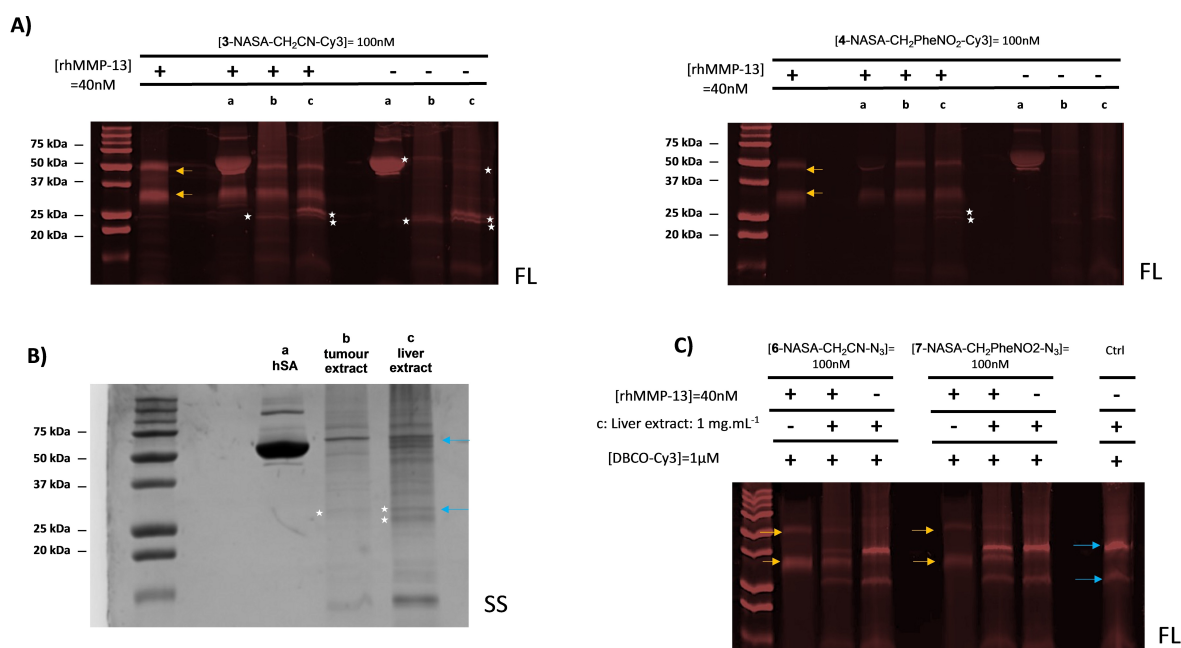


Figure 3. Labeling of recombinant hMMP-13 in complex proteomes. A) In-gel fluorescence analysis of hMMP-13 (40 nM, 0.2% (w/w) of the whole proteome) artificially introduced in complex proteomes (a: human serum albumin, b: mouse tumor extract, and c: mouse liver extract) at 1 g/L and labeled by either probe **3** (left panel) or **4** (right panel) used at 100 nM. The labeling reaction was conducted over 2 h at 37 °C. All samples were subjected to 15% SDS-PAGE and imaged by in-gel fluorescence imaging (FL). The two fluorescent bands corresponding to Cy3-labeled MMP-13 are marked with orange arrow. Off targets labeling is marked with white stars. B) Silver staining (SS) of the three different proteomes. C) In-gel fluorescence analysis of hMMP-13 (40 nM, 0.2% (w/w) of the whole proteome) spiked into mouse liver extract (1 g/L), and labeled by either probe **6** and **7** used at 100 nM. The labeling reaction was conducted over 2 h at 37 °C, followed by a blocking reaction with iodo acetamide (1 mM) for 30 min at room temperature and a strained-promoted cyclo addition reaction with Cy3-DBCO conducted at 4 °C during 12 h. Each sample was then diluted with 4X Laemmli buffer without reductants, heated for 5 min at 95 °C, resolved by 15% SDS-PAGE and in-gel fluorescence imaged. Fluorescent bands due to the unspecific formation of protein conjugates with DBCO-Cy3 are marked with blue arrows.

active form of MMP, thus confirming their mode of action as activity-based probes. The identification of the covalently labelled residue within the hMMP-13 catalytic cleft further support this selective reactivity. In complex proteomes, reducing the reactivity of the NASA motif proves particularly critical, with the less reactive probe 4 displaying both good labeling selectivity toward the targeted MMP and limited unspecific reactivity. Notably, probe 4 hardly reacts with human serum albumin, whereas probe 3 significantly modifies this protein. Interestingly, part of this unspecific reactivity seems to be attributed to the fluorescent reporter itself, which is consistent with previous observations made not only in the phosphinic pseudo-peptides series^[31,32] but also with other small-molecule probes.^[33,34] In this regard, when the Cy3 moiety is replaced by a simple azido tag, the resulting probes (probes 6 and 7) behave quite similarly in complex proteomes. Overall, and in line with recent findings,^[35] our study demonstrates the importance of finely controlling the reactivity of the NASA warhead to improve the selectivity of covalent probes in complex biological systems.

Conclusions

We have designed and validated a new generation of MMP-directed AfBPs with both an optimal MMP labeling profile and a reduced nonspecific reactivity. We clearly demonstrated that a wide range of MMPs could be efficiently labeled by small-molecule probes targeting their S₃ subsite. Importantly, the observed covalent modification occurred only on the active forms of MMPs, confirming these probes as true activity-based probes. In this series, we also showed that reducing the electrophilicity of the NASA cleavable linker significantly impacted both the reactivity and specificity of the probes. This aspect is particularly critical in complex proteomes, where the less reactive AfBP appeared to be the most selective towards the targeted MMP. These optimized MMP-directed probes are currently being studied in complex biological matrices containing active forms of endogenous MMPs and could prove useful in profiling the activation status of MMPs in various mouse models, an area that remains unaddressed to date.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Metalloproteases · Affinity-based probes · Ligand-directed chemistry · N-acyl-N-alkyl sulfonamide cleavable linker

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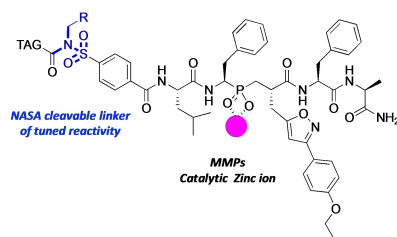
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RESEARCH ARTICLE

Original affinity-based probes with a N-acyl-N-alkyl sulfonamide (NASA) cleavable linker were designed to covalently modify several human Matrix Metalloproteases (MMPs). The electrophilicity of the cleavable linker was optimized to reduced unspecific reactivity of the probes in complex biological matrices. The probes, tested in varied proteomes containing MMP-13, showed improved selectivity and reduced off-target propensity.



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Covalent Labeling of Matrix Metalloproteases with Affinity-Based Probes Containing Tuned Reactive N-Acyl-N-Alkyl Sulfonamide Cleavable Linkers

