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Competitive profiling of ligandable cysteines in *Staphylococcus aureus* with an organogold compound†

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With the idea of exploiting metal templated C–S bond forming reactions to achieve modification of cysteines in bacterial proteins, a cyclometalated Au(III) compound was explored in a competitive chemoproteomic approach in *S. aureus* cell extracts. More than 100 ligandable cysteines were identified, of which more than 50% were not engaged by organic α-chloroacetamides in a previous study, indicating that organometallic compounds expand the ligandable space in bacteria. A selected interaction was validated using an enzyme activity assay, and intact protein mass spectrometry showed cysteine arylation of an unprecedented target. The obtained results demonstrate that this family of organogold compounds has potential for therapeutic protein targeting *via* selective, covalent modification of cysteine residues in bacteria.

Bacterial antimicrobial resistance is one of the leading causes of death world-wide and is estimated to have directly caused more than 1.2 million deaths in 2019.[1] One way to overcome this global health challenge will be the identification of novel antibacterial targets that can be liganded with small molecules.[2] While recent successes with organic compounds as reversible binders show that there is still a lot of potential to address new targets with known concepts,[3–5] systematically exploring other compound classes for antibacterial activity could open up entirely new target families. In this context, metal complexes possess many attractive properties as novel therapeutic candidates addressing elusive target proteins especially for antibiotic applications.[6–9] This is based on the unique 3D metallodrugs' structures, inaccessible to organic molecules,[6,10] as well as on the ability of metal complexes to undergo activation by ligand exchange reactions and/or redox mechanisms in cells.[11,12] Notably, organometallic compounds featuring a direct metal–carbon bond have attracted increasing attention as 'catalytic drugs', since they also promote bioorthogonal transformations in biological environment.[13]

Recently, some of us started working on various organometallic Au(III) complexes, which were shown to possess anticancer and antibacterial properties.[14–19] Specifically, we focused on cyclometalated Au(III) C^N complexes and their protein targets.[11,19,20] It was observed that organogold compounds in this family can template the formation of covalent C–S bonds at cysteine residues *via* cross-coupling.[21–25] In this series, the [Au(C^CO^N)Cl$_2$] compound (**1**, C^CO^N = 2-benzoylpyridine, Fig. 1a) was identified as the most reactive and prone to cysteine arylation (Fig. 1b) in buffered aqueous solution (pH 7.4) at 37 °C.[21] Furthermore, **1** showed moderate antibacterial effects (MIC approx. 12.5–50 μM, unpublished data and ref. 19) *in vitro*. Thus, we envisaged the application of compounds of this family as (i) tools for competitive residue-specific chemoproteomic technologies to enable profiling of unprecedented ligandable cysteine residues in bacterial cells,[26–28] and (ii) as promising novel cysteine-targeted antibacterial agents.

Recently, we demonstrated that irreversible, cysteine-directed covalent binders in combination with competitive, residue-specific chemoproteomic approaches allow efficient identification of many new ligandable cysteines in bacteria in parallel using the isoDTB–ABPP (isotopically labelled desthiobiotin azide-activity-based protein profiling) technology,[28] which is based on the isoTOP–ABPP (isotopic tandem orthogonal proteolysis–ABPP) platform.[26,27] Using isoDTB–ABPP, we started investigating in an unbiased fashion, which cysteines can be engaged with organogold compounds and eventually subjected to arylation in the entire *S. aureus* proteome. To avoid laborious synthesis of Au(III) C^N complexes with suitable

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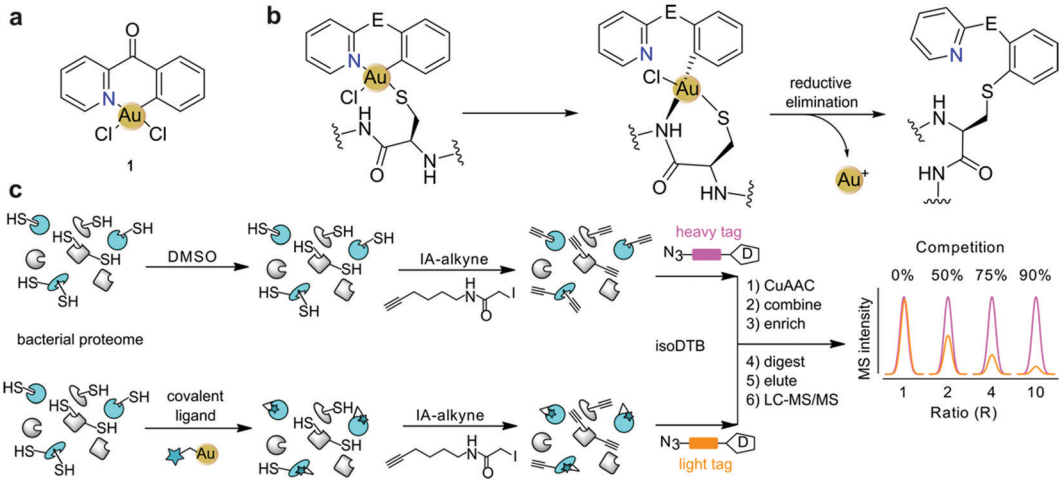
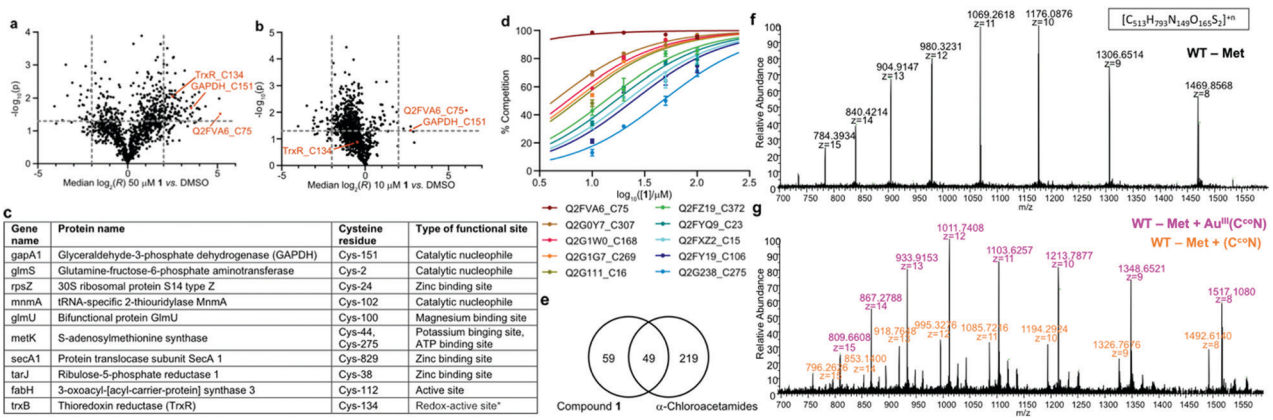


Fig. 1 (a) Structure of the cyclometalated complex [Au(C^CO^N)Cl$_2$] (**1**, C^CO^N = 2 benzoylpyridine). (b) Proposed mechanism of cysteine arylation templated by compound **1** *via* reductive elimination. (c) Workflow of isoDTB–ABPP experiments. isoDTB: isotopically labeled affinity tags, D: desthiobiotin.

affinity handles, we applied a competitive approach (Fig. 1c).[28] The latter has already been applied for the Au(I) complex auranofin as a (seleno-)cysteine electrophile.[29]

In brief, in this strategy, the *S. aureus* proteome is split into two samples. One is treated with compound **1** and the other one with DMSO as a solvent control. In the next step, iodoacetamide alkyne (IA-alkyne)[26] is used to label many cysteine residues with alkynes. At the sites, at which compound **1** is already bound, this reactivity is blocked leading to a difference in alkyne modification between the inhibitor-treated and control-treated sample. This difference is read out using isotopically labelled (light and heavy) desthiobiotin azide (isoDTB) tags that are appended by copper-catalyzed azide–alkyne cycloaddition (CuAAC)[30] and after combination of the two samples used for enrichment. Following digestion of the enriched proteins, the modified peptides are eluted and quantified by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). While cysteines that are not bound by **1** will show ratios between the heavy and light channel close to $R = 1$, the specific targets of **1** will show high ratios ($R \gg 1$).

As the modified peptides are directly detected, this technology not only allows determination of the target proteins, but also of the exact interaction sites. In this way, a global understanding of the binding sites that are ligandable by compound **1** is obtained in the entire *S. aureus* proteome. Following this chemoproteomic approach, bacterial cell extracts were treated



Gene name	Protein name	Cysteine residue	Type of functional site
gapA1	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Cys-151	Catalytic nucleophile
glmS	Glutamine-fructose-6-phosphate aminotransferase	Cys-2	Catalytic nucleophile
rpsZ	30S ribosomal protein S14 type Z	Cys-24	Zinc binding site
mnmA	tRNA-specific 2-thiouridylase MnmA	Cys-102	Catalytic nucleophile
glmU	Bifunctional protein GlmU	Cys-100	Magnesium binding site
metK	S-adenosylmethionine synthase	Cys-44, Cys-275	Potassium binging site, ATP binding site
secA1	Protein translocase subunit SecA 1	Cys-829	Zinc binding site
tarJ	Ribulose-5-phosphate reductase 1	Cys-38	Zinc binding site
fabH	3-oxoacyl-[acyl-carrier-protein] synthase 3	Cys-112	Active site
trxB	Thioredoxin reductase (TrxR)	Cys-134	Redox-active site*

Fig. 2 (a and b) Volcano plots of the isoDTB–ABPP experiments that show the median $\log_2(R)$ of the ratio between the light (compound-treated) and heavy (DMSO-treated) channels and the $-\log_{10}(p)$ of the statistical significance in a one-sample *t*-test for all quantified cysteines for compound **1** at 50 μM (a) and 10 μM (b). Grey dotted lines indicate the cut-offs of $\log_2(R) = \pm 2$ and $p < 0.05$ that were used for hit selection. Selected proteins discussed in the text are highlighted in orange. (c) Cysteines liganded by **1** at 50 μM or below that are in annotated functional sites of proteins encoded by essential genes. *: Functional site inferred from the 100% identical gene with Uniprot code P66011. (d) Concentration dependence of the degree of competition determined using isoDTB-ABPP for a selection of cysteines. Data points represent the median, error bars the standard deviation and lines a dose–response curve fit. All experiments were performed in duplicate. (e) Venn diagram of the ligandable cysteines identified in this study with compound **1** (based on the cysteines ligandable by **1** in Table S1, ESI†) and in a previous study with a library of α-chloroacetamides (ligandable cysteines listed in Table S4, ESI of that study).[28] (f) HR-ESI-MS spectrum of WT GCN5-like putative *N*-acetyltransferase and (g) spectrum after addition of **1** incubated for 1 h at rt.

with compound **1** at room temperature for 1 hour at concentrations ranging from 10 μM to 100 μM. It should be noted that **1** is only moderately active as antibacterial agent on *S. aureus* culture (MIC values approx. 50 μM).[19] Therefore, we deprioritized the identification of targets in living cells at low concentrations and rather focused on the broad mapping of cysteines that are ligandable with this compound class in lysates at higher concentrations. Using the isoDTB–ABPP platform, we obtained data on a total of 1486 cysteines in the *S. aureus* proteome (Table S1, ESI†). While at 100 μM broad competition at many targets was observed (Fig. S1, ESI†), we detected a much narrower window of specifically competed proteins at 50 μM and below (Fig. 2a, and b and Fig. S1, ESI†). For further analysis, we focussed on the proteins that were significantly engaged ($\log_2(R) > 2$, $p < 0.05$) at 50 μM or below. Thus, we identified 108 cysteines that are ligandable by compound **1**. Interestingly, 27 of them were found in proteins encoded by essential genes[31] and, of those, 10 were assigned to be close to the respective functional protein sites (Fig. 2c).[32] The latter include catalytic nucleophiles (Cys-151 in gapA1, Cys-2 in glmS and Cys-102 of mnmA), other active site residues (Cys-134 in trxB and Cys-112 in fabH), residues of metal binding sites (Cys-24 of rpsZ, Cys-100 of glmU, Cys-829 in secA1 and Cys-38 in tarJ) and residues of nucleotide binding sites (Cys-44 and Cys-275 in metK).[32]

In line with typical organic molecules as competitors,[33] we detected concentration-dependent competition for many cysteines (Fig. 2d) indicating that the isoDTB–ABPP method gives quantitative, residue-specific engagement data also for organogold compounds. Moreover, of the 108 cysteines liganded by compound **1**, 59 were not liganded by any member of a previously screened α-chloroacetamide library (Fig. 2e, Table S1, ESI†).[28] These unique targets include Cys-44 of metK, Cys-24 of rpsZ and Cys-829 in secA1, located in functional sites of proteins encoded by essential genes (Fig. 2c). These results indicate that organometallic compounds like **1** indeed access a different portion of the proteome and can, therefore, be very beneficial to target binding sites that are hard to address with organic compounds.

Of note, one of the competed cysteines was located in the functional site of the protein encoded by gapA1, corresponding to Glyceraldehyde-3-phosphate dehydrogenase (GADPH), an enzyme recently unveiled as a target of antibacterial Ag^+ ions in *E. coli*, and inhibited by Cu^+ in *S. aureus*.[34,35]

Moreover, the results highlighted Cys-134 of the bacterial thioredoxin reductase (TrxR, Fig. 2a and c) as one targeted residue. TrxR belongs to the antioxidant thioredoxin system[36,37] and features a redox active disulfide/dithiol couple (Cys-134 and Cys-137). Interestingly, TrxR has already been proposed as pharmacological target for antibacterial gold compounds.[38–40] To assess the effect of compound **1** on enzyme activity, we conducted inhibition studies with purified *S. aureus* TrxR using a DTNB-based assay.[38] In accordance with the isoDTB–ABPP data, compound **1** efficiently inhibits TrxR activity (IC_{50} = 0.258 ± 0.052 μM, Fig. S2, ESI†).

As competition of IA-alkyne labelling in isoDTB–ABPP experiments, as well as TrxR inhibition, is in principle possible by either coordination of the Au(III) centre to the target cysteine or by the aforementioned C–S cross-coupling reaction, we set out to validate the actual mechanism-of-action on one of our identified target proteins, namely the GCN5-like putative *N*-acetyltransferase (Uniprot code Q2FVA6). While this protein is not encoded by an essential gene, it stood out by its high engagement even at 10 μM of compound **1** (R = 67 at 10 μM corresponding to >98% competition). At this concentration, **1** showed high selectivity having only four other targets with significantly lower R values (Fig. 2b). It should be noted that Cys-75 is not liganded by any α-chloroacetamide in our earlier study (Table S1, ESI†),[28] showing that this is an interaction that is specific to the gold complex **1**. Additionally, in a published NMR structure,[41] Cys-75 is close to the thioester of the cofactor acetyl CoA (3.3 Å, Fig. S3a, ESI†) indicating that blocking it with compound **1** has the potential to inhibit the enzyme activity.

The relatively small size of this protein (approx. 11.9 kDa) allowed us to perform intact protein high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) studies following previously reported procedures.[21,42] The wild type protein (WT) and its mutant (Mu, C75A) were incubated with compound **1** (5 μM) in 1 : 3 ratio in H_2O/ACN (2 : 1) for 1 h at rt. The obtained results (Fig. 2f, g and Fig. S3 and S4, ESI†) demonstrate the formation of the Au(III) coordination adduct [WT-Met + $Au^{III}(C^{CO}N)]^{n+}$ obtained upon exchange of the two chlorido ligands of **1**, as well as the cysteine arylation product [WT-Met + $(C^{CO}N)]^{n+}$ (Fig. 2g, and Fig. S3, Tables S2 and S3, ESI†). These results are in line with previously reported reactivity studies of compound **1** and derivatives with model peptides.[20,21,24] Of note, the unbound WT protein species could not be identified in the gold-treated sample indicating the protein's marked reactivity with **1**. In the case of the C75A mutant, only formation of species featuring a bound gold fragment of general formula [Mu-Met + $Au^{III}(C^{CO}N)]^{n+}$ were identified, while the signals of the unbound protein were still detected (Fig. S4, Tables S2 and S3, ESI†). As expected, the arylation product could not be observed in this experiment. Inhibition of the enzymatic activity of TrxR as well as arylation of Cys-75 of GCN5-like putative *N*-acetyltransferase validate the chemoproteomic data and verify that arylation with compound **1** can proceed on unprecedented proteins identified using this technology.

Here, we have successfully profiled cysteines in the pathogenic bacterium *S. aureus* that can be liganded with the organogold compound **1** using the isoDTB–ABPP technology. Our study shows that Au(III) cyclometalated compounds have a high potential to address unique binding sites in the proteome, where they can promote selective metal-templated reactions and thereby elicit biological effects that are not attainable with organic molecules in a straightforward fashion. Despite these promising results in bacterial lysates, compound **1** shows only moderate antibacterial activity against *S. aureus.* This could be due to different factors, including its extracellular deactivation, low uptake into bacterial cells or an insufficiently high engagement of the relevant cysteines in the complex cell environment. Therefore, further optimization of the compound's scaffold will be necessary in the future, for example including targeting functionalities in the C^N backbone.[22,43] It is worth mentioning that, in a recent study,

some of us reported on a cyclometalated Au(III) C^N analogue of **1** with promising antibacterial effects,[19] that is now undergoing target engagement studies at relevant concentrations in living bacteria.

As many metal complexes that could potentially bind to or react selectively with cysteine residues have been reported to have antibacterial activity,[6] this study opens up the field of quantitatively studying their cellular interactions using residue-specific chemoproteomics, provided that the bonding interactions will be sufficiently stable. Through performance of such experiments in bacteria to identify relevant targets, as well as in human cell lines to identify potential off-targets, we envision that this technology will make significant contributions to realising the potential of metal complexes as antibacterial drugs in the near future.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1 Antimicrobial Resistance Collaborators, *Lancet*, 2022, **399**, 629–655.
2 M. Lakemeyer, W. Zhao, F. A. Mandl, P. Hammann and S. A. Sieber, *Angew. Chem., Int. Ed.*, 2018, **57**, 14440–14475.
3 J. K. Martin, II, J. P. Sheehan, B. P. Bratton, G. M. Moore, A. Mateus, S. H.-J. Li, H. Kim, J. D. Rabinowitz, A. Typas, M. M. Savitski, M. Z. Wilson and Z. Gitai, *Cell*, 2020, **181**, 1518–1532.e14.
4 J. M. Stokes, K. Yang, K. Swanson, W. Jin, A. Cubillos-Ruiz, N. M. Donghia, C. R. MacNair, S. French, L. A. Carfrae, Z. Bloom-Ackermann, V. M. Tran, A. Chiappino-Pepe, A. H. Badran, I. W. Andrews, E. J. Chory, G. M. Church, E. D. Brown, T. S. Jaakkola, R. Barzilay and J. J. Collins, *Cell*, 2020, **180**, 688–702.e13.
5 P. Le, E. Kunold, R. Macsics, K. Rox, M. C. Jennings, I. Ugur, M. Reinecke, D. Chaves-Moreno, M. W. Hackl, C. Fetzer, F. A. M. Mandl, J. Lehmann, V. S. Korotkov, S. M. Hacker, B. Kuster, I. Antes, D. H. Pieper, M. Rohde, W. M. Wuest, E. Medina and S. A. Sieber, *Nat. Chem.*, 2020, **12**, 145–158.
6 A. Frei, J. Zuegg, A. G. Elliott, M. Baker, S. Braese, C. Brown, F. Chen, C. G. Dowson, G. Dujardin, N. Jung, A. P. King, A. M. Mansour, M. Massi, J. Moat, H. A. Mohamed, A. K. Renfrew, P. J. Rutledge, P. J. Sadler, M. H. Todd, C. E. Willans, J. J. Wilson, M. A. Cooper and M. A. T. Blaskovich, *Chem. Sci.*, 2020, **11**, 2627–2639.
7 B. Đ. Glišić and M. I. Djuran, *Dalton Trans.*, 2014, **43**, 5950–5969.
8 M. B. Harbut, C. Vilchèze, X. Luo, M. E. Hensler, H. Guo, B. Yang, A. K. Chatterjee, V. Nizet, W. R. Jacobs, Jr., P. G. Schultz and F. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 4453–4458.
9 H. Wang, M. Wang, X. Xu, P. Gao, Z. Xu, Q. Zhang, H. Li, A. Yan, R. Y.-T. Kao and H. Sun, *Nat. Commun.*, 2021, **12**, 3331.
10 J. Hess, *Biol. Chem.*, 2021, **403**, 363–375.
11 A. de Almeida, B. L. Oliveira, J. D. G. Correia, G. Soveral and A. Casini, *Coord. Chem. Rev.*, 2013, **257**, 2689–2704.
12 A. Casini, A. Guerri, C. Gabbiani and L. Messori, *J. Inorg. Biochem.*, 2008, **102**, 955–1006.
13 J. Rodríguez and M. Martínez-Calvo, *Chem. – Eur. J.*, 2020, **26**, 9792–9813.
14 B. Bertrand and A. Casini, *Dalton Trans.*, 2014, **43**, 4209–4219.
15 B. Bertrand, S. Spreckelmeyer, E. Bodio, F. Cocco, M. Picquet, P. Richard, P. Le Gendre, C. Orvig, M. A. Cinellu and A. Casini, *Dalton Trans.*, 2015, **44**, 11911–11918.
16 S. Spreckelmeyer, M. van der Zee, B. Bertrand, E. Bodio, S. Stürup and A. Casini, *Front. Chem.*, 2018, 6.
17 M. V. Babak, K. R. Chong, P. Rapta, M. Zannikou, H. M. Tang, L. Reichert, M. R. Chang, V. Kushnarev, P. Heffeter, S. M. Meier-Menches, Z. C. Lim, J. Y. Yap, A. Casini, I. V. Balyasnikova and W. H. Ang, *Angew. Chem., Int. Ed.*, 2021, **60**, 13405–13413.
18 A. Pintus, M. C. Aragoni, M. A. Cinellu, L. Maiore, F. Isaia, V. Lippolis, G. Orrù, E. Tuveri, A. Zucca and M. Arca, *J. Inorg. Biochem.*, 2017, **170**, 188–194.
19 P. Chakraborty, D. Oosterhuis, R. Bonsignore, A. Casini, P. Olinga and D.-J. Scheffers, *ChemMedChem*, 2021, **16**, 3060–3070.
20 M. N. Wenzel, S. M. Meier-Menches, T. L. Williams, E. Rämisch, G. Barone and A. Casini, *ChemComm*, 2018, **54**, 611–614.
21 M. N. Wenzel, R. Bonsignore, S. R. Thomas, D. Bourissou, G. Barone and A. Casini, *Chem. – Eur. J.*, 2019, **25**, 7628–7634.
22 K. K.-Y. Kung, H.-M. Ko, J.-F. Cui, H.-C. Chong, Y.-C. Leung and M.-K. Wong, *ChemComm*, 2014, **50**, 11899–11902.
23 R. E. F. de Paiva, Z. Du, D. H. Nakahata, F. A. Lima, P. P. Corbi and N. P. Farrell, *Angew. Chem., Int. Ed.*, 2018, **57**, 9305–9309.
24 S. R. Thomas, R. Bonsignore, J. Sánchez Escudero, S. M. Meier-Menches, C. M. Brown, M. O. Wolf, G. Barone, L. Y. P. Luk and A. Casini, *ChemBioChem*, 2020, **21**, 3071–3076.
25 S. R. Thomas and A. Casini, *Curr. Opin. Chem. Biol.*, 2020, **55**, 103–110.
26 E. Weerapana, C. Wang, G. M. Simon, F. Richter, S. Khare, M. B. D. Dillon, D. A. Bachovchin, K. Mowen, D. Baker and B. F. Cravatt, *Nature*, 2010, **468**, 790–795.
27 K. M. Backus, B. E. Correia, K. M. Lum, S. Forli, B. D. Horning, G. E. Gonzalez-Paez, S. Chatterjee, B. R. Lanning, J. R. Teijaro, A. J. Olson, D. W. Wolan and B. F. Cravatt, *Nature*, 2016, **534**, 570–574.
28 P. R. A. Zanon, L. Lewald and S. M. Hacker, *Angew. Chem., Int. Ed.*, 2020, **59**, 2829–2836.
29 D. W. Bak, J. Gao, C. Wang and E. Weerapana, *Cell Chem. Biol.*, 2018, **25**, 1157–1167.e4.
30 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
31 R. R. Chaudhuri, A. G. Allen, P. J. Owen, G. Shalom, K. Stone, M. Harrison, T. A. Burgis, M. Lockyer, J. Garcia-Lara, S. J. Foster, S. J. Pleasance, S. E. Peters, D. J. Maskell and I. G. Charles, *BMC Genomics*, 2009, **10**, 291.
32 The UniProt Consortium, *Nucleic Acids Res.*, 2021, **49**, D480–D489.
33 K. M. Lee, P. Le, S. A. Sieber and S. M. Hacker, *ChemComm*, 2020, **56**, 2929–2932.
34 H. Wang, M. Wang, X. Yang, X. Xu, Q. Hao, A. Yan, M. Hu, R. Lobinski, H. Li and H. Sun, *Chem. Sci.*, 2019, **10**, 7193–7199.
35 E. Tarrant, G. P. Riboldi, M. R. McIlvin, J. Stevenson, A. Barwinska-Sendra, L. J. Stewart, M. A. Saito and K. J. Waldron, *Metallomics*, 2019, **11**, 183–200.
36 C. H. Williams Jr, L. D. Arscott, S. Müller, B. W. Lennon, M. L. Ludwig, P.-F. Wang, D. M. Veine, K. Becker and R. H. Schirmer, *Eur. J. Biochem.*, 2000, **267**, 6110–6117.
37 E. S. J. Arnér and A. Holmgren, *Eur. J. Biochem.*, 2000, **267**, 6102–6109.
38 C. Schmidt, B. Karge, R. Misgeld, A. Prokop, R. Franke, M. Brönstrup and I. Ott, *Chem. – Eur. J.*, 2017, **23**, 1869–1880.
39 C. Schmidt, B. Karge, R. Misgeld, A. Prokop, M. Brönstrup and I. Ott, *MedChemComm*, 2017, **8**, 1681–1689.
40 R. Büssing, B. Karge, P. Lippmann, P. G. Jones, M. Brönstrup and I. Ott, *ChemMedChem*, 2021, **16**, 3402–3409.
41 J. R. Cort, T. A. Ramelot, D. Murray, T. B. Acton, L.-C. Ma, R. Xiao, G. T. Montelione and M. A. Kennedy, *J. Struct. Funct. Genomics*, 2008, **9**, 7–20.
42 M. Wenzel and A. Casini, *Coord. Chem. Rev.*, 2017, **352**, 432–460.
43 S. Gukathasan, S. Parkin, E. P. Black and S. G. Awuah, *Inorg. Chem.*, 2021, **60**, 14582–14593.