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# "Click" disaggregation-induced emission of a fluorescent dye<sup>+</sup>

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Here we demonstrate a new approach to fluorogenic labelling, where a cationic hemicyanine (CHyC) exhibits disaggregationinduced emission (DIE) upon undergoing an azide-alkyne "click" reaction. CHyC self-associates and is self-quenched in aqueous buffer over a low micromolar concentration range. When an azido nucleoside (AmdU) or azide-containing cellular DNA is added to CHyC in the presence of Cu(i), a copper-catalysed azide-alkyne cycloaddition drives dye disaggregation, significantly increasing the fluorescence intensity of the probe upon its covalent attachment to modified biomolecules.

Fluorogenic bioorthogonal "click" chemical reactions can enable convenient, no-wash cellular imaging.<sup>1</sup> In the context of nucleic acids,<sup>2</sup> click reactions with fluorescent probes provide powerful tools for characterizing DNA/RNA metabolism, cell cycle progression, viral entry, and therapeutic mechanisms of known and new drug candidates.<sup>3</sup> Classical fluorophores like rhodamines, cyanines, coumarins, and others<sup>4</sup> are now widely available with clickable handles—such as tetrazines, azides, and alkynes—to facilitate conjugation reactions such as copper-catalysed azide–alkyne cycloadditions (CuAAC).<sup>5</sup> Increasing the fluorescence intensity of the labelled biomolecule as compared to the unreacted dye is an important and challenging goal in wash-free imaging applications.<sup>6</sup>

Cyanine dyes are a diverse family of fluorophores which are classified by the number of methine "bridge" units and terminal heterocycles present.<sup>7</sup> Styryl hemicyanines containing two methine carbons have been used in three-way junction DNA aptamers,<sup>8</sup> fluorescent oligonucleotide probes,<sup>9</sup> and for noncovalent binding of DNA.<sup>10</sup> Moreover, the metabolic modification of nucleic acids with alkene groups followed by treatment with tetrazine-substituted styryl hemicyanines enabled inverse electron-demand Diels–Alder (IEDDA) reactions on cellular DNA.<sup>11</sup> Indeed, tetrazines are well established to quench fluorophores,<sup>12</sup> allowing for wash-free imaging of metabolically labelled DNA in live cells.<sup>6b</sup>

Azides groups are invaluable in chemical biology and drug development due to their small size and bioorthogonal reactivity.13 Despite their widespread applications,<sup>3f,14</sup> a general "turn-on" strategy for azide-reactive dyes remains elusive. Azide-alkyne cycloadditions are not inherently fluorogenic, although triazole formation has been shown to result in increased in emissions of highly tailored systems.<sup>15</sup> Exploring innovative turn-on mechanisms for azide-modified nucleic acids, such as disaggregationinduced emission (DIE) where fluorescence is triggered by the disaggregation of aggregated probes is a promising new approach (Scheme 1).16 Non-covalent DIE reactions have previously been used for detecting small molecules,<sup>17</sup> monitoring the equilibrium of G-quadruplexes,<sup>18</sup> and probing cellular membranes and proteins.<sup>19</sup> Herein, we designed a cationic hemicyanine (CHyC) that exhibits DIE upon reacting with an azide-containing nucleoside, 5-(azidomethyl)-2'-deoxyuridine (AmdU),14d via CuAAC reaction. The irreversible covalent chemical reaction shifts the dye selfassociation equilibrium towards disaggregation, resulting in enhanced fluorescence emission.

To synthesize CHyC, 6-methoxy-2-naphthaldehyde 1 was transformed into benzoindole 2 through a base-promoted



Scheme 1 A quenched and aggregated alkyne-containing fluorescent dye undergoes disaggregation and enhanced fluorescence upon CuAAC reaction with azido DNA.

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Scheme 2 Synthesis of CHyC (5) and all relevant intermediates where EtOH = ethanol, DMF = N,N-dimethylformamide, LAH = lithium aluminium hydride, THF = tetrahydrofuran, and ACN = acetonitrile. See the ESI† for the synthesis and characterization of these compounds.

Knoevenagel condensation and Hemetsberger indolization (Scheme 2).<sup>20</sup> First, ethyl-2-azidoacetate 1a was synthesized in a 98% yield from ethyl-2-bromoacetate.<sup>21</sup> 6-Methoxy-2-naphthaldehyde 1 and azidoacetate 1a were dissolved in ethanol along with a sacrificial electrophile, ethyl trifluoroacetate. 20% sodium ethoxide in ethanol was added at 0 °C and the reaction was stirred overnight yielding the  $\alpha$ -azido- $\beta$ -arylacrylate **1b**. Thermolysis of intermediate **1b** gave the benzo[g]indole 2 as the only regioisomeric indole in a 62% yield over two-steps. The propargyl group was introduced by treating 2 with sodium hydride followed by the dropwise addition of propargyl bromide to give the desired product 3 in an 83% yield. 3 was then reduced to the corresponding aldehyde 4 in two consecutive steps in a 79% yield. 4 and 1,2,3,3-tetramethyl-3H-indol-1-ium iodide were heated to 70 °C overnight in ethanol to yield CHyC 5 as a dark purple solid with low water solubility in 91% isolated yield (Scheme 2). The probe and all relevant intermediates were fully characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and high-resolution ESI MS (see ESI<sup>+</sup>). Stock solutions of CHyC for photophysical and biological studies were prepared in DMSO and diluted into the indicated solvents (0.5% DMSO unless stated otherwise) prior to analysis.

The photophysical properties of CHyC 5 were evaluated at various concentrations upon dilution into  $1 \times$  PBS buffer, pH = 7.4 (Fig. 1a). The aqueous samples displayed a linear relationship between absorbance ( $\lambda_{max}$  = 520 nm) and CHyC concentration over the range 0.2–12.4  $\mu$ M (ESI,† Fig. S1,  $\varepsilon_{520}$  = 32 300 cm<sup>-1</sup> M<sup>-1</sup>). In contrast, non-linear concentrationdependent effects were observed in the fluorescence emission intensities of the same samples (ESI,† Fig. S1), giving lower quantum yield values ( $\phi = 1.1-0.063\%$ ) with increasing concentration (Fig. 1b). Microscopic evaluation of the samples prepared at 2-10 µM in PBS revealed the presence of purple, nonfluorescent particles with diameters ranging from roughly 2-8 µm (ESI,† Fig. S2). In contrast, CHyC samples prepared entirely in DMSO exhibited better solubility, a higher measured extinction coefficient ( $\varepsilon_{545}$  = 41 900 cm<sup>-1</sup> M<sup>-1</sup>) and concentrationindependent quantum yield ( $\Phi$  = 5.4%). In DMSO, CHyC



Fig. 1 (a) Absorbance (dashed) and fluorescence (solid) spectra of  $0.2-12.4 \,\mu$ M solutions of CHyC 5 in 1× PBS (pH 7.4, 2% EtOH). (b) Calculated quantum yields verses CHyC concentrations in 1× PBS. (c) Absorbance (dashed) and fluorescence (solid) spectra of a 2  $\mu$ M solution of CHyC 5 in various solvents and 1× PBS (pH 7.4, 2% EtOH). (d) CuAAC reaction of CHyC 5 and AmdU where THPTA = tris(benzyltriazolylmethyl)amine. (e) Fluorescence spectrum of a 100  $\mu$ M solution of CHyC, 1 mM CuSO<sub>4</sub>, 2 mM THPTA, 1 mM AmdU, and 10 mM sodium ascorbate in PBS pH 7.4 (1.3% DMSO) at time = 0 min, 20 min, 40 min, and 60 min into the reaction. For all fluorescence: ex: 546 nm, em: 570–750 nm. See the ESI† for the characterization of CHyC-AmdU-triazole 6.

exhibited a red-shifted absorbance ( $\lambda_{max} = 540 \text{ nm}$ ) and emission ( $\lambda_{max} = 625 \text{ nm}$ ) as compared to 1× PBS. The absorbance spectrum of CHyC in acetonitrile (ACN) closely resembled that of DMSO. However, in methanol (MeOH), additional solvent effects led to a further redshift of CHyC, albeit with a lower quantum yield ( $\Phi = 1.0\%$ ) than the 5.4% for DMSO (Fig. 1c and ESI,† Table S1). Together these results suggest that the microaggregated form(s) of CHyC in PBS have some twisting about the styryl bridge and/or self-assembly into H-type aggregates.<sup>22</sup> The dynamic, self-quenching and self-association behaviour of CHyC over the low  $\mu$ M concentration range suggested that it may exhibit "turn-on" fluorescence behaviour upon chemical reaction with groups that would endow enhanced solubility properties of the product in water.

To evaluate if a click reaction involving a partially soluble dye can induce disaggregation-induced emission (DIE), a 100  $\mu$ M solution of CHyC 5 was subjected to standard CuAAC conditions with a 10-fold excess of AmdU in 1× PBS containing 1% DMSO (Fig. 1d). The reaction was monitored by fluorescence (Fig. 1e) as well as high performance liquid chromatography (ESI,† Fig. S3). Both analyses indicated complete consumption of CHyC 5 in less than one hour. Remarkably, the fluorescence intensity of the solution showed a ~3-fold increase; reminiscent of the changes observed in DMSO (Fig. 1c). The CHyC-AmdU-triazole reaction product 6 was isolated in a 70% yield and was characterized to confirm its identity (see ESI†). These results demonstrate that DIE during a bioorthogonal chemical reaction can be used to track reaction progress in real time.

To evaluate the potential utility of DIE of CHyC in no-wash cellular staining and imaging, HeLa cell cultures were treated with 100 µM of an AmdU monophosphate derivative bearing two 5'pivaloyloxymethyl masking groups "POM-AmdU",<sup>23</sup> for 17 hours prior to fixation and staining with 10  $\mu$ M CHyC in 1 $\times$  PBS containing 1% DMSO and Cu(I). The cells were imaged while still in the staining solution, revealing large fluorescence enhancements of the nuclei in cells pre-treated with POM-AmdU as compared to those receiving vehicle only. As a control, we compared the performance of CHyC with a commercially available Cy5 alkyne derivative "Alexa Fluor™ 647 Alkyne" that was also found to be compatible with no-wash imaging, but it displayed little or no selectivity for the cellular nuclei of cells that had been pre-treated with POM-AmdU (ESI,† Fig. S4). To evaluate the DNA selectivity of CHyC staining in POM-AmdU treated cells, the CHyC staining solutions were removed by aspiration, and a second solution containing the non-covalent DNA stain Hoechst 33342 was added to the cells and imaged without washing (Fig. 2). Only cells receiving POM-AmdU exhibited CHyC "turn-on" fluorescence that co-localized with Hoechst staining with a Pearson correlation coefficient (PCC) of 0.76  $\pm$  0.03 as compared to a PCC = 0.31  $\pm$ 0.08 for the control cells not pre-treated with POM. A perfect correlation of 1.0 was not expected because only a fraction of the cells had passed though S-phase during the 17-hour incubation with POM-AmdU.

In summary, CHyC is a novel cationic hemicyanine dye that undergoes disaggregation-induced emission (DIE) after CuAAC click reactions. In the current example, DNA is targeted by



Fig. 2 Visualization of azide-modified DNA in HeLa cells treated with 100  $\mu$ M of POM-AmdU for 17 hours followed by fixation and no-wash CuAAC staining with 10  $\mu$ M CHyC **5** in the presence of 1 mM CuSO<sub>4</sub>, 2 mM THPTA, and 10 mM sodium ascorbate for 2 hours. The CuAAC solution was aspirated without washing, and Hoechst 33342 was used added as nuclear co-stain and directly imaged. Negative control samples received no POM-AmdU but were otherwise treated identically.

virtue of AmdU incorporation into cellular DNA. In theory, RNA could be targeted by CHyC by using appropriate metabolic labels such as  $N^6$ -ethylazido-adenosine or 2'-azidoadenosine.<sup>14f</sup> While fast, the CuAAC reaction is limited to fixed cells due to its toxicity,<sup>24</sup> and hence catalyst-free DIE reactions based on SPAAC<sup>25</sup> or vinyl-tetrazine ligation<sup>6b</sup> could provide future access to wash-free imaging of live cells.

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#### Data availability

The data supporting this article have been included in the main article and as part of the ESI.†

### Conflicts of interest

There are no conflicts to declare.

#### Notes and references

- 1 Y. Chen, H. Jiang, T. Hao, N. Zhang, M. Li, X. Wang, X. Wang, W. Wei and J. Zhao, *Chem. Biomed. Imaging*, 2023, **1**(7), 590–619.
- 2 (a) D. Ganz, D. Harijan and H.-A. Wagenknecht, *RSC Chem. Biol.*, 2020, 1(3), 86–97; (b) N. Z. Fantoni, A. H. El-Sagheer and T. Brown, *Chem. Rev.*, 2021, 121(12), 7122–7154; (c) J. I. H. Knaack and C. Meier, *ChemMedChem*, 2024, 19(15), e202400160.

- 3 (a) S. Ding, X. Qiao, J. Suryadi, G. S. Marrs, G. L. Kucera and U. Bierbach, Angew. Chem., Int. Ed., 2013, 52(12), 3350–3354; (b) A. B. Neef, L. Pernot, V. N. Schreier, L. Scapozza and N. W. Luedtke, Angew. Chem., Int. Ed., 2015, 54(27), 7911–7914; (c) T. Triemer, A. Messikommer, S. M. K. Glasauer, J. Alzeer, M. H. Paulisch and N. W. Luedtke, Proc. Natl. Acad. Sci. U. S. A., 2018, 115(7), E1366–E1373; (d) M. Tera, Z. Harati Taji and N. W. Luedtke, Angew. Chem., 2018, 130(47), 15631–15635; (e) M. Kubota, S. Nainar, S. M. Parker, W. England, F. Furche and R. C. Spitale, ACS Chem. Biol., 2019, 14(8), 1698–1707; (f) A. Messikommer, K. Seipel, S. Byrne, P. J. M. Valk, T. Pabst and N. W. Luedtke, ACS Pharmacol. Transl. Sci., 2020, 3(6), 1225–1232; (g) Y. Li, Y. Ling, M. O. Loehr, S. Chaabane, O. W. Cheng, K. Zhao, C. Wu, M. Buscher, J. Weber, D. Stomakhine, M. Munker, R. Pientka, S. B. Christ, M. Dobbelstein and N. W. Luedtke, Life Sci., 2023, 330, 122000.
- 4 N. Klöcker, F. P. Weissenboeck and A. Rentmeister, *Chem. Soc. Rev.*, 2020, **49**(23), 8749–8773.
- 5 S. L. Scinto, D. A. Bilodeau, R. Hincapie, W. Lee, S. S. Nguyen, M. Xu, C. W. Am Ende, M. G. Finn, K. Lang, Q. Lin, J. P. Pezacki, J. A. Prescher, M. S. Robillard and J. M. Fox, *Nat. Rev. Methods Primers*, 2021, 1, 30.
- 6 (a) J. B. Grimm, B. P. English, J. Chen, J. P. Slaughter, Z. Zhang, A. Revyakin, R. Patel, J. J. Macklin, D. Normanno, R. H. Singer, T. Lionnet and L. D. Lavis, *Nat. Methods*, 2015, 12(3), 244–250; (b) M. O. Loehr and N. W. Luedtke, *Angew. Chem., Int. Ed.*, 2022, e202112931; (c) A. Spampinato, E. Kuzmova, R. Pohl, V. Sykorova, M. Vrabel, T. Kraus and M. Hocek, *Bioconjugate Chem.*, 2023, 34(4), 772–780; (d) V. T. Sterrenberg, D. Stalling, J. I. H. Knaack, T. K. Soh, J. B. Bosse and C. Meier, *Angew. Chem., Int. Ed.*, 2023, 62(38), e202308271; (e) M. Kuba, P. Khoroshyy, M. Lepsik, E. Kuzmova, D. Kodr, T. Kraus and M. Hocek, *Angew. Chem., Int. Ed.*, 2023, 62(38), e202307548; (f) A. Martin and P. Rivera-Fuentes, *Nat. Chem.*, 2024, 16(1), 28–35.
- 7 (a) A. Mishra, R. K. Behera, P. K. Behera, B. K. Mishra and G. B. Behera, *Chem. Rev.*, 2000, **100**(6), 1973–2011; (b) G. S. Gopika, P. M. H. Prasad, A. G. Lekshmi, S. Lekshmypriya, S. Sreesaila, C. Arunima, M. S. Kumar, A. Anil, A. Sreekumar and Z. S. Pillai, *Mater. Today Proc.*, 2021, **46**, 3102–3108.
- 8 (a) A. J. Van Riesen, J. Le, S. Slavkovic, Z. R. Churcher, A. A. Shoara, P. E. Johnson and R. A. Manderville, ACS Appl. Bio Mater., 2021, 4(9), 6732–6741; (b) A. J. Van Riesen, B. Kalnitsky, A. A. Shoara, S. Slavkovic, Z. R. Churcher, P. E. Johnson and R. A. Manderville, Dyes Pigm., 2023, 209.
- 9 (a) M. M. Rubner, C. Holzhauser, P. R. Bohländer and H. A. Wagenknecht, Chem. Eur. J., 2012, 18(5), 1299–1302; (b) S. Arndt, H.-K. Walter and H.-A. Wagenknecht, J. Vis. Exp., 2016, 113, e54121; (c) J. Steinmeyer, F. Rönicke, U. Schepers and H. A. Wagenknecht, ChemistryOpen, 2017, 6(4), 514–518; (d) B. Ditmangklo, J. Taechalertpaisarn, K. Siriwong and T. Vilaivan, Org. Biomol. Chem., 2019, 17(45), 9712–9725; (e) J. Gebhard, L. Hirsch, C. Schwechheimer and H. A. Wagenknecht, Bioconjugate Chem., 2022, 33(9), 1634–1642; (f) P. Geng, E. List, F. Rönicke and H. A. Wagenknecht, Chem. Eur. J., 2023, 29(8), e202203156.
- 10 (a) P. R. Bohländer and H.-A. Wagenknecht, Org. Biomol. Chem., 2013, 11(43), 7458; (b) K. Supabowornsathit, K. Faikhruea, B. Ditmangklo, T. Jaroenchuensiri, S. Wongsuwan, S. Junpra-Ob, I. Choopara, T. Palaga, C. Aonbangkhen, N. Somboonna, J. Taechalertpaisarn and T. Vilaivan, Sci. Rep., 2022, 12, 14250; (c) S. Wangngae, U. Ngivprom, T. Khrootkaew, S. Worakaensai, R.-Y. Lai and A. Kamkaew, RSC Adv., 2023, 13(3), 2115–2122.
- (a) D. Ganz, P. Geng and H. A. Wagenknecht, ACS Chem. Biol., 2023, 18(5), 1054–1059; (b) B. Pfeuffer, P. Geng and H. A. Wagenknecht, ChemBioChem, 2024, 25(4), e202300739; (c) N. Seul, D. Lamade,

P. Stoychev, M. Mijic, R. T. Michenfelder, L. Rieger, P. Geng and

- H. A. Wagenknecht, Angew. Chem., Int. Ed., 2024, 63(22), e202403044.
- 12 H. Wu, J. Yang, J. Seckute and N. K. Devaraj, Angew. Chem., Int. Ed., 2014, 53(23), 5805–5809.
- 13 F. Müggenburg and S. Müller, *Chem. Rec.*, 2022, **22**(5), e202100322.
- 14 (a) S. Broder, Antivir. Res., 2010, 85(1), 1–18; (b) M. Aigner, M. Hartl, K. Fauster, J. Steger, K. Bister and R. Micura, ChemBioChem, 2011, 12(1), 47–51; (c) A. H. El-Sagheer and T. Brown, Chem. Commun., 2011, 47(44), 12057; (d) A. B. Neef and N. W. Luedtke, ChemBioChem, 2014, 15(6), 789–793; (e) J. M. Holstein, D. Schulz and A. Rentmeister, Chem. Commun., 2014, 50(34), 4478–4481; (f) S. Nainar, S. Beasley, M. Fazio, M. Kubota, N. Dai, I. R. Corrêa and R. C. Spitale, ChemBioChem, 2016, 17(22), 2149–2152; (g) M. K. Yates and K. L. SeleyRadtke, Antiviral Res., 2019, 162, 5–21; (h) L. Taemaitree, A. Shivalingam, A. H. El-Sagheer and T. Brown, Nat. Commun., 2019, 10, 1610; (i) D. Wang, Y. Zhang and R. E. Kleiner, J. Am. Chem. Soc., 2020, 142(34), 14417–14421; (j) S. Moreno, J. M. Ramos Pittol, M. Hartl and R. Micura, Org. Biomol. Chem., 2022, 20(39), 7845–7850.
- (a) Z. Zhou and C. J. Fahrni, J. Am. Chem. Soc., 2004, 126(29), 8862;
  (b) J. C. Jewett and C. R. Bertozzi, Org. Lett., 2011, 13(22), 5937–5939;
  (c) P. Shieh, M. J. Hangauer and C. R. Bertozzi, J. Am. Chem. Soc., 2012, 134(42), 17428–17431;
  (d) F. Friscourt, C. J. Fahrni and G. J. Boons, J. Am. Chem. Soc., 2012, 134(45), 18809–18815.
- 16 (a) D. Zhai, W. Xu, L. Zhang and Y.-T. Chang, *Chem. Soc. Rev.*, 2014, 43(8), 2402; (b) K. Saczuk, M. Dudek, K. Matczyszyn and M. Deiana, *Nanoscale Horiz.*, 2024, 9, 1390–1416.
- (*a*) L. K. Kumawat, A. A. Abogunrin, M. Kickham, J. Pardeshi, O. Fenelon, M. Schroeder and R. B. P. Elmes, *Front. Chem.*, 2019, 7; (*b*) P. Zhang, M. S. Zhu, H. Luo, Q. Zhang, L. E. Guo, Z. Li and Y. B. Jiang, *Anal. Chem.*, 2017, **89**(11), 6210–6215; (*c*) J. J. Gao, X. X. Lang, Q. Q. Yu, H. Y. Li, H. J. Wang and M. Q. Wang, *Spectrochim. Acta, Part A*, 2021, **252**, 119492; (*d*) L. Liu, C. Liu, L. Wang, X.-C. Shen and H. Chen, *Sens. Actuators, B*, 2022, **371**, 132542.
- 18 (a) M. Deiana, K. Chand, J. Jamroskovic, I. Obi, E. Chorell and N. Sabouri, Angew. Chem., Int. Ed., 2020, 59(2), 896–902; (b) M. Deiana, K. Chand, J. Jamroskovic, R. N. Das, I. Obi, E. Chorell and N. Sabouri, Nanoscale, 2020, 12(24), 12950–12957; (c) S. Liu, L. Bu, Y. Zhang, J. Yan, L. Li, G. Li, Z. Song and J. Huang, Anal. Chem., 2021, 93(12), 5267–5276; (d) G.-F. Liu, Y.-S. Chen, Z.-L. Wang, D. Gu and M.-Q. Wang, Dyes Pigm., 2024, 225, 112107.
- (a) K. Mizusawa, Y. Ishida, Y. Takaoka, M. Miyagawa, S. Tsukiji and I. Hamachi, J. Am. Chem. Soc., 2010, 132(21), 7291–7293; (b) T.-C. Hou, Y.-Y. Wu, P.-Y. Chiang and K.-T. Tan, Chem. Sci., 2015, 6(8), 4643–4649; (c) D. Wu, S. Cheung, G. Sampedro, Z. L. Chen, R. A. Cahill and D. F. O'Shea, Biochim. Biophys. Acta, Biomembr., 2018, 1860(11), 2272–2280; (d) J.-Z. Li, H.-L. Lin, H.-Y. Li, H.-W. Cao, X.-X. Lang, Y.-S. Chen, H.-W. Chen and M.-Q. Wang, Dyes Pigm., 2023, 216, 111357.
- 20 W. L. Heaner Iv, C. S. Gelbaum, L. Gelbaum, P. Pollet, K. W. Richman, W. Dubay, J. D. Butler, G. Wells and C. L. Liotta, RSC Adv., 2013, 3(32), 13232.
- 21 F. Shi, J. P. Waldo, Y. Chen and R. C. Larock, *Org. Lett.*, 2008, **10**(12), 2409–2412.
- 22 (a) M. Kasha, Radiat. Res., 1963, 20(1), 55–70; (b) A. S. Klymchenko, J. Nanosci. Lett., 2013, 3(21), 1–8.
- 23 M. Tera, S. M. K. Glasauer and N. W. Luedtke, *ChemBioChem*, 2018, **19**(18), 1939–1943.
- 24 J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**(43), 16793–16797.
- 25 M. Tera and N. W. Luedtke, Methods Enzymol., 2020, 641, 433-457.