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"Click" disaggregation-induced emission of a fluorescent dye†

Kaleena Basran[a] and Nathan W. Luedtke *[ab]

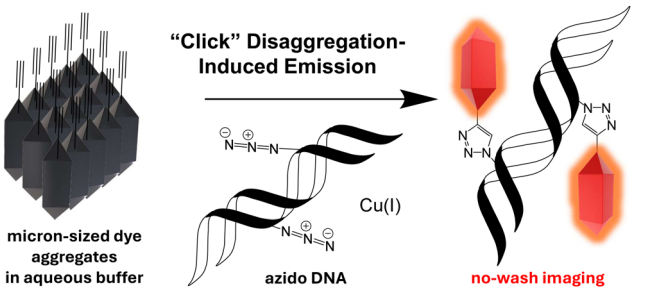
Here we demonstrate a new approach to fluorogenic labelling, where a cationic hemicyanine (CHyC) exhibits disaggregation-induced emission (DIE) upon undergoing an azide–alkyne "click" reaction. CHyC self-associates and is self-quenched in aqueous buffer over a low micromolar concentration range. When 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.[1] In the context of nucleic acids,[2] 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.[3] Classical fluorophores like rhodamines, cyanines, coumarins, and others[4] 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).[5] 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.[6]

Cyanine dyes are a diverse family of fluorophores which are classified by the number of methine "bridge" units and terminal heterocycles present.[7] Styryl hemicyanines containing two methine carbons have been used in three-way junction DNA aptamers,[8] fluorescent oligonucleotide probes,[9] and for non-covalent binding of DNA.[10] 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.[11] Indeed, tetrazines are well established to quench fluorophores,[12] allowing for wash-free imaging of metabolically labelled DNA in live cells.[6b]

Azides groups are invaluable in chemical biology and drug development due to their small size and bioorthogonal reactivity.[13] Despite their widespread applications,[3f,14] 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.[15] Exploring innovative turn-on mechanisms for azide-modified nucleic acids, such as disaggregation-induced 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,[17] monitoring the equilibrium of G-quadruplexes,[18] and probing cellular membranes and proteins.[19] 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 self-association 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.

[a] *Department of Chemistry, McGill University, Montreal, Quebec H3A 0B8, Canada*
[b] *Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3G 1Y6, Canada*

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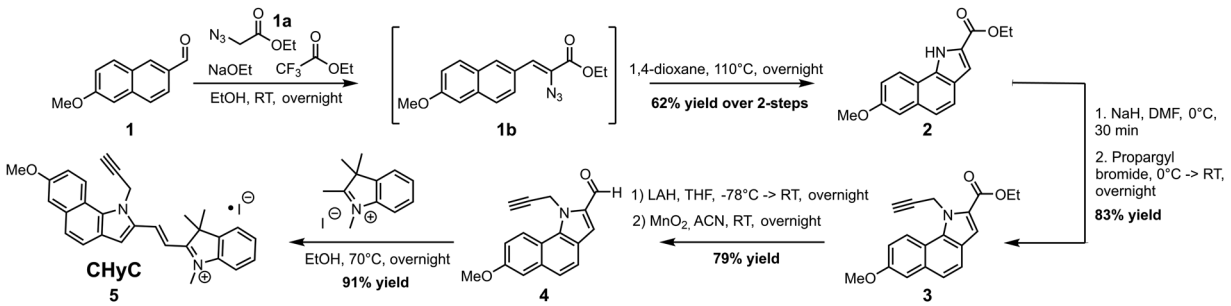

Scheme 2 Synthesis of CHyC (**5**) and all relevant intermediates where EtOH = ethanol, DMF = *N*,*N*-dimethylformamide, LAH = lithium aluminium hydride, THF = tetrahydrofuran, and ACN = acetonitrile. See the ESI† for the synthesis and characterization of these compounds.

Knoevenagel condensation and Hemetsberger indolization (Scheme 2).[20] First, ethyl-2-azidoacetate **1a** was synthesized in a 98% yield from ethyl-2-bromoacetate.[21] 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 α-azido-β-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-3*H*-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 ^{1}H NMR, ^{13}C NMR, and high-resolution ESI MS (see ESI†). 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× PBS buffer, pH = 7.4 (Fig. 1a). The aqueous samples displayed a linear relationship between absorbance (λ_{max} = 520 nm) and CHyC concentration over the range 0.2–12.4 μM (ESI,† Fig. S1, ε_{520} = 32 300 cm^{-1} M^{-1}). In contrast, non-linear concentration-dependent effects were observed in the fluorescence emission intensities of the same samples (ESI,† Fig. S1), giving lower quantum yield values (Φ = 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, non-fluorescent 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 (ε_{545} = 41 900 cm^{-1} M^{-1}) and concentration-independent quantum yield (Φ = 5.4%). In DMSO, CHyC

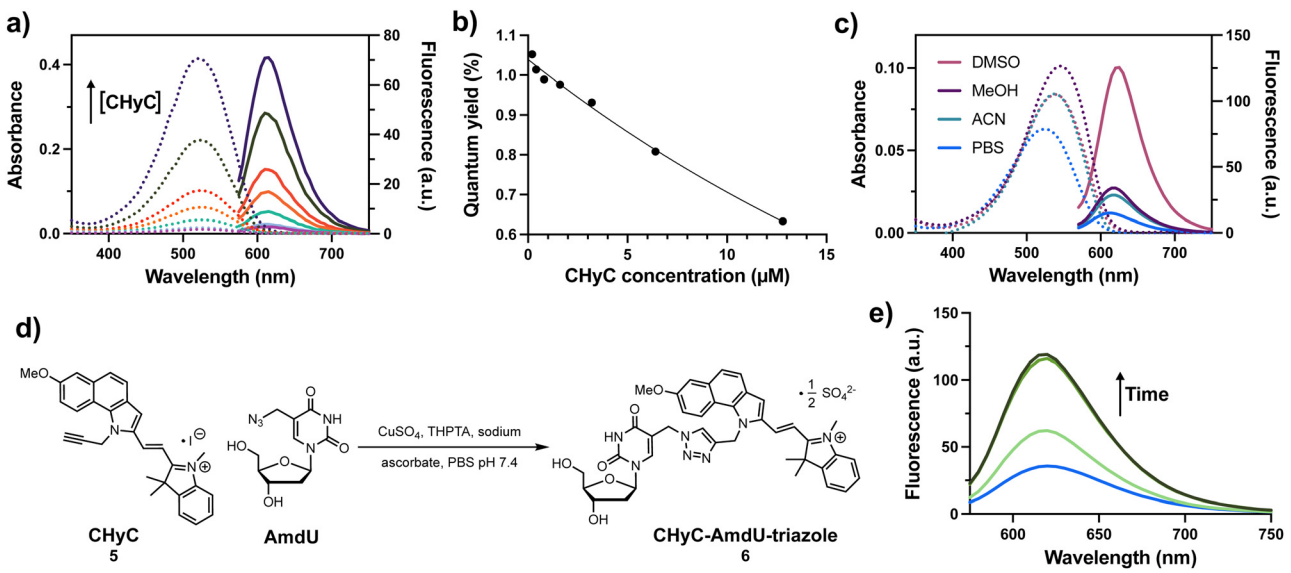

Fig. 1 (a) Absorbance (dashed) and fluorescence (solid) spectra of 0.2–12.4 μM solutions of CHyC **5** in 1× PBS (pH 7.4, 2% EtOH). (b) Calculated quantum yields verses CHyC concentrations in 1× PBS. (c) Absorbance (dashed) and fluorescence (solid) spectra of a 2 μM solution of CHyC **5** in various 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 μM solution of CHyC, 1 mM $CuSO_4$, 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 (λ_{max} = 540 nm) and emission (λ_{max} = 625 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 (Φ = 1.0%) than the 5.4% for DMSO (Fig. 1c and ESI,† Table S1). Together these results suggest that the micro-aggregated form(s) of CHyC in PBS have some twisting about the styryl bridge and/or self-assembly into H-type aggregates.[22] The dynamic, self-quenching and self-association behaviour of CHyC over the low μM 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 μM 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",[23] for 17 hours prior to fixation and staining with 10 μM CHyC in 1× 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 ± 0.03 as compared to a PCC = 0.31 ± 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 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.[14f] While fast, the CuAAC reaction is limited to fixed cells due to its toxicity,[24] and hence catalyst-free DIE reactions based on SPAAC[25] or vinyl-tetrazine ligation[6b] could provide future access to wash-free imaging of live cells.

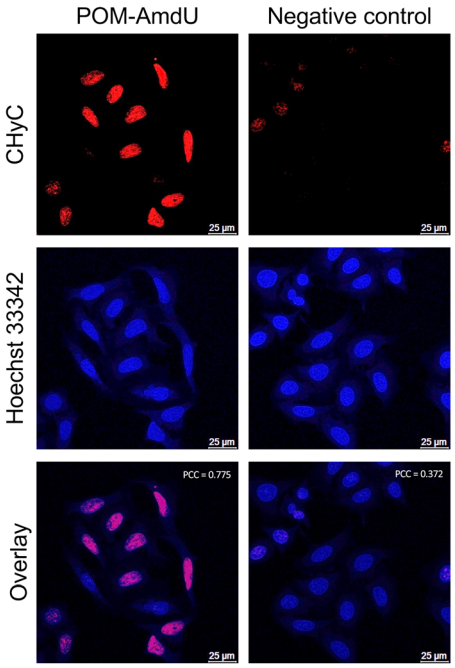

Fig. 2 Visualization of azide-modified DNA in HeLa cells treated with 100 μM of POM-AmdU for 17 hours followed by fixation and no-wash CuAAC staining with 10 μM CHyC **5** in the presence of 1 mM $CuSO_4$, 2 mM THPTA, and 10 mM sodium ascorbate for 2 hours. The CuAAC solution was aspirated without washing, 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.

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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.
11 (*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. Seley-Radtke, *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.
15 (*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.
17 (*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.
19 (*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.