reaction fulfils all of the click criteria, it has become the poster child for click chemistry. It was the first click reaction to be widely adopted, and is now used in applications spanning many disciplines, from materials science to chemical biology[6,7].

Several other click reactions have emerged over the past few years. Of particular note is one known as sulfur(VI)–fluoride exchange (SuFEx), which links an oxygen or nitrogen atom to an SO_2F group. SuFEx is generally recognized as a second category of click reaction[8,9] (unlike other click reactions, it is not a cycloaddition process), and has been used in a diverse range of chemical transformations[9,10].

Despite the power of CuAAC reactions, their applications would be even broader if structurally complex, azide-containing compounds were more widely available. Conventionally, organic azides are synthesized by replacing a molecular fragment called a leaving group with an azide group; the leaving group can be a variety of chemical groups or just a single atom. However, the azide anions used in these substitution reactions are highly nucleophilic (electron-rich) and therefore very reactive. Substitutions with azide anions are thus often incompatible with having other chemical groups in the molecule. Furthermore, the leaving group often needs to be made in advance from an alcohol group (OH), which can be difficult or impossible to achieve selectively on molecules that contain many chemical groups.

Alternatively, azides can be prepared from primary amines (compounds that contain NH_2 groups) by a 'diazotransfer' reaction. Until now, the state-of-the-art reagent used to carry out diazotransfer had been trifluoromethanesulfonyl azide[11] ($CF_3SO_2N_3$). However, the reactions often require an excess of this reagent, are slow, and do not always proceed to completion, with 60–70% as the typical yield.

Meng *et al.* have addressed these limitations by developing a more efficient diazotransfer reagent, fluorosulfuryl azide (FSO_2N_3). They report that it reacts with almost any primary amine in a one-to-one ratio, achieving a nearly 100% yield of the corresponding azide. The authors demonstrated the reagent's substrate scope and practicality by using it to make a library of 1,224 azides in 96-well plates. It is notable that 49% of these azides had not been synthesized before, according to the authors' literature search.

The number of azides synthesized is impressive (see Supplementary Information Section 6 of the paper[1] for a full list), but the most striking aspect of this study is the substrate scope: the reaction works for different amine subclasses, on complex molecules, and in the presence of various chemical groups. Moreover, Meng and colleagues' diazotransfer reaction meets the speed, breadth and efficiency criteria for click chemistry.

In addition, the authors demonstrated that the prepared azide solutions can be used directly in CuAAC reactions. This opens the door to a highly efficient and general two-step method for converting primary amines — a common chemical group in organic molecules — into triazoles. Notably, this method does not require the amines to be modified in advance to prevent unwanted side reactions at other chemical groups; nor does it require the intermediate azides to be purified.

Triazoles are functional mimics of the amide bond[12], which is found in many pharmaceutical agents and in all proteins. Triazoles can also function as surrogates for sugars in polysaccharides[13]. Meng and co-workers' chemistry could therefore be used to synthesize well-characterized libraries of complex small molecules and biomacromolecules from readily available precursors. More broadly, the work brings us a step closer to the vision laid out by the pioneers of click chemistry[3,5]: the development of a few operationally simple reactions that use common precursors to rapidly generate diverse libraries of (bio)molecules that have desirable functional properties. ■

Joseph J. Topczewski *and* **En-Chih Liu** *are in the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, USA.*
e-mail: jtopczew@umn.edu

1. Meng, G. *et al. Nature* **574**, 86–89 (2019).
2. Campos, K. R. *et al. Science* **363**, eaat0805 (2019).
3. Kolb, H. C., Finn, M. G. & Sharpless, K. B. *Angew. Chem. Int. Edn* **40**, 2004–2021 (2001).
4. Rostovtsev, V. V., Green, L. G., Fokin, V. V. & Sharpless, K. B. *Angew. Chem. Int. Edn* **41**, 2596–2599 (2002).
5. Tornøe, C. W., Christensen, C. & Meldal, M. *J. Org. Chem.* **67**, 3057–3064 (2002).
6. Meldal, M. & Tornøe, C. W. *Chem. Rev.* **108**, 2952–3015 (2008).
7. Hein, J. E. & Fokin, V. V. *Chem. Soc. Rev.* **39**, 1302–1315 (2010).
8. Dong, J., Krasnova, L., Finn, M. G. & Sharpless, K. B. *Angew. Chem. Int. Edn* **53**, 9430–9448 (2014).
9. Barrow, A. S. *et al. Chem. Soc. Rev.* **48**, 4731–4758 (2019).
10. Schimler, S. D. *et al. J. Am. Chem. Soc.* **139**, 1452–1455 (2017).
11. Cavender, C. J. & Shiner, V. J. *J. Org. Chem.* **37**, 3567–3569 (1972).
12. Pedersen, D. S. & Abell, A. *Eur. J. Org. Chem.* **2011**, 2399–2411 (2011).
13. Tiwari, V. K. *et al. Chem. Rev.* **116**, 3086–3240 (2016).

NEUROSCIENCE

A daily rhythm in colour preference

Behavioural and genetic experiments have revealed that fruit flies prefer green light over other colours in the morning and evening, and always avoid blue. These colour preferences rely on different mechanisms. SEE LETTER P.108

CHARLOTTE HELFRICH-FÖRSTER

Colour vision helps animals to find nutritious food, to avoid poisonous animals and, in some cases, in social interactions[1]. Colour can affect people's mood, and their colour preferences might reflect current emotional and physiological states[2]. Colour preferences also seem to vary through the seasons[3]. Lazopulo *et al.*[4] show on page 108 that the fruit fly *Drosophila* avoids blue light, and prefers green light to red light at different times of the daily 24-hour cycle. The authors also pinpoint separate mechanisms for these behavioural responses.

Light influences various behaviours in insects, and fruit flies serve as a model in which to study the mechanisms underlying this effect. Flies avoid or are attracted to light depending on its intensity and colour and the duration and time of day of the exposure[5,6]. However, it is unclear whether fruit flies have intrinsic colour preferences, as do primates[7] and, if so, how these preferences are mediated.

Lazopulo *et al.* analysed video recordings of the position and movements of individual flies living in glass tubes, each of which contained three equally sized zones that were covered by blue, green or red filters. The authors placed food at one end of each tube, and varied the order of the coloured zones along the tubes to avoid misinterpreting flies' preference for the zone that contained food as reflecting a colour preference. To simulate the day–night cycle, the flies were kept in light–dark conditions (12 hours of light and 12 hours of darkness), and thus the colours were visible only during the light phase. Consistent with this, the flies showed no preference for any particular coloured part of the tube during the dark phase.

During the light phase, however, the flies exhibited a complex, systematic pattern of colour preference (Fig. 1). They consistently avoided the blue-light zone; furthermore, they spent more time in the green zone than in the red zone in the early morning and late afternoon, when the flies showed bursts of activity[8]. Such timed preferences are intuitively advantageous, because some of this activity is devoted to searching for food, and flies often find food in or under green trees and bushes.

Flies lack photoreceptors (light-sensitive cells) that are specifically sensitive to red light, although their green-light photoreceptors show some sensitivity to red light[9]; thus, they

probably experience the red light as dim green light or just dim light. Lazopulo *et al.* observed that the flies tended to rest under red or green light during their midday 'siesta' (Fig. 1). However, when they could choose between bright green light and dim white light, the flies generally chose to have their siesta in dim white light. These results suggest that flies prefer to rest under dim light.

Nonetheless, when the flies could choose between bright green and dim blue light for their siesta, the flies always preferred green. This implies that flies decide between green and blue on the basis of colour (wavelength) of the light, rather than intensity. Indeed, their strong aversion to blue light during the siesta makes sense because blue light (and ultraviolet light, which has a slightly shorter wavelength and higher energy) can damage DNA and can therefore be harmful[10].

Light-activated pigments called rhodopsins are expressed by photoreceptor cells in the eyes of animals. The authors monitored the colour preferences of fly strains that were genetically engineered to have mutations in genes expressed by photoreceptor cells, including those encoding rhodopsins. They found that rhodopsin 1, which is the most abundant rhodopsin expressed in the compound eyes of fruit flies, is necessary for green-light preference, whereas rhodopsin 6 contributes to the preference for dim light during siesta time. Both rhodopsins are more sensitive to green light than to other colours, and photoreceptor cells that express these rhodopsins signal to a group of neuronal cells in the brain — known as the central clock[11] — that regulate many biological processes over the 24-hour (circadian) period. Thus, the central clock might time the changes in green-light preference.

'Clock' genes expressed by neurons in the central clock interact with one another in a complex feedback loop that serves as a timekeeping mechanism. The authors tested clock-gene mutants[12] whose genetic clock did not function, or ran faster or slower than normal. Flies that lacked a functioning clock did not show morning and evening peaks in green-light preference and instead preferred green light throughout the entire light period. By contrast, mutants whose internal clocks ran faster or slower than normal had an abnormally timed preference to green light. Notably, however, the clock-gene mutants still avoided blue light, indicating that this behaviour does not require a functional circadian clock.

Unexpectedly, the authors established that the photoreceptor cells responsible for blue-light avoidance are not located in the head; neither the compound eyes, nor the blue-light-sensitive protein cryptochrome, which is present in the circadian clock neurons, were needed for this response. Instead, rhodopsin 7, expressed by pain-sensing neurons of the body wall, was required for blue-light avoidance. These neurons mediate escape responses of the fly to high temperatures, potentially harmful chemicals and mechanical stimuli. Rhodopsin 7 is present at low levels in several fly tissues, and its sensitivity to ultraviolet and blue light was characterized only a few years ago[13,14]. Its function has been debated, but Lazopulo *et al.* seem to have found a crucial function of rhodopsin 7.

The study shows for the first time that pain-sensing neurons in the body wall of adult flies can detect light and mediate consistent avoidance of short-wavelength light. Until now, this had been reported only in larvae[15]. Previous studies measured adult flies' colour preferences by placing them between two tubes lit with different colours (generally UV, green or blue)[5,16,17]. Depending on the available choice, the flies moved towards UV or blue light. By contrast, the flies in Lazopulo and colleagues' study were not attracted to blue or UV light at any time of the day.

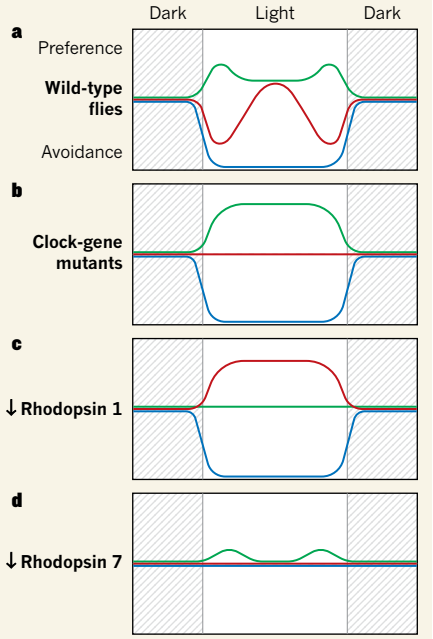

Figure 1 | Flies show differences in colour preference during the day. Lazopulo *et al.*[4] assessed the colour preferences of fruit flies (*Drosophila*) at various times of day as they moved freely between three differently coloured zones — blue, red and green — of glass tubes. **a**, Wild-type flies showed no preference for any of the three zones during the 12-hour dark period. But, during the light phase, they avoided the blue zone and preferred the green zone to the red zone during their morning and evening activity bouts. **b**, Flies that were genetically engineered to lack a 24-hour biological rhythm (clock-gene mutants) preferred green light to red light across the whole light phase. **c**, By contrast, flies that expressed less of a light-sensitive pigment called rhodopsin 1 in their eyes than normal flies did not prefer green light to red light (which flies perceive as dim light). **d**, Unexpectedly, flies that expressed less-than-normal levels of another rhodopsin pigment, rhodopsin 7, in pain-sensitive neuronal cells in the body wall did not avoid blue light.

This difference in result is probably due to the fact that in the Lazopulo *et al.* experimental set-up, the flies could freely choose a preferred place, completely undisturbed. In previous studies, flies were aroused either by being placed together in a new environment[5,16,17] or by acute exposure to light[6] before they could choose between different colours. Therefore, these previous experiments measured phototactic responses — that is, movements towards or away from a light source — rather than intrinsic preferences for certain colours. Phototactic responses depend on the function of the flies' eyes[5,6,16,17]. Thus, the mechanisms that control phototactic behaviour and the avoidance of blue light are distinct.

It remains to be seen how the two pathways that control the preference for green light and the avoidance of blue light work together in fruit flies. Lazopulo and colleagues' findings also raise the question of how other animals, including mammals, respond to different colours at different times of day, and whether similar pathways exist in other animals. Rhodopsin 7 is present in most arthropods[18] (joint-legged invertebrates) and might have a similar role in these animals. However, it is absent in vertebrates, and humans don't seem to have a sensor in their skin that enables them to avoid blue light. If they did, they probably wouldn't spend hours in the sun getting a tan. ■

Charlotte Helfrich-Förster *is in the Department of Neurobiology and Genetics, Theodor Boveri Institute, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany.*
e-mail: charlotte.foerster@biozentrum.uni-wuerzburg.de

1. Osorio, D. & Vorobyev, M. *Vision Res.* **48**, 2042–2051 (2008).
2. Whitfield, T. W. & Wiltshire, T. J. *Genet. Soc. Gen. Psychol. Monogr.* **116**, 385–411 (1990).
3. Schloss, K. B., Nelson, R., Parker, L., Heck, I. A. & Palmer, S. E. *Cogn. Sci.* **41**, 1589–1612 (2017).
4. Lazopulo, S., Lazopulo, A., Baker, J. D. & Syed, S. *Nature* **574**, 108–111 (2019).
5. Yamaguchi, S., Desplan, C. & Heisenberg, M. *Proc. Natl. Acad. Sci. USA* **107**, 5634–5639 (2010).
6. Baik, L. S., Recinos, Y., Chevez, J. A., Au, D. D. & Holmes, T. C. *J. Biol. Rhythms* **34**, 391–400 (2019).
7. Wells, D. L., McDonald, C. L. & Ringland, J. E. *J. Comp. Psychol.* **122**, 213–219 (2008).
8. Hamblen-Coyle, M. J., Wheeler, D. A., Rutila, J. E., Rosbash, M. & Hall, J. C. *J. Insect Behav.* **5**, 417–446 (1992).
9. Salcedo, E. *et al. J. Neurosci.* **19**, 10716–10726 (1999).
10. Kielbassa, C., Roza, L. & Epe, B. *Carcinogenesis* **18**, 811–816 (1997).
11. Alejevski, F. *et al. Nature Commun.* **10**, 252 (2019).
12. Konopka, R. J. & Benzer, S. *Proc. Natl Acad. Sci. USA* **68**, 2112–2116 (1971).
13. Ni, J. D., Baik, L. S., Holmes, T. C. & Montell, C. *Nature* **545**, 340–344 (2017).
14. Senthilan, P. R., Grebler, R., Reinhard, N., Rieger, D. & Helfrich-Förster, C. *Biology* **8**, 6 (2019).
15. Xiang, Y. *et al. Nature* **468**, 921–926 (2010).
16. Heisenberg, M. & Buchner, E. *J. Comp. Physiol.* **117**, 127–162 (1977).
17. Gao, S. *et al. Neuron* **60**, 328–342 (2008).
18. Senthilan, P. R. & Helfrich-Förster, C. *PeerJ* **4**, e2427 (2016).

This article was published online on 18 September 2019.