contributes to the cellular sensitivity to camptothecin.

A unique aspect of the Flor et al. article is the mechanism by which topoisomerase I-mediated DNA cleavage is induced. Because the presence of DNA lesions generated by reactive oxygen species and lipid peroxidation products is known to increase topoisomerase I- and II-mediated DNA strand breaks, it has been proposed that cleavage enhancement induced by oxidative stress is triggered by genomic damage (Pommier and Osheroff, 2011). However, Flor et al. provide evidence for an alternative mechanism. The authors demonstrate that 4-hydroxy-2-nonenal forms a covalent adduct with topoisomerase I, modifying cysteine 630 by a Michael addition. Thus, this is the first report of a *covalent* topoisomerase I poison. A variety of reactive quinones, polyphenols, and isothiocyanates have been identified as covalent topoisomerase II poisons (Ketron and Osheroff, 2014). These compounds adduct cysteine residues in the type II enzyme that are outside of the DNA cleavage-ligation active site and are believed to enhance DNA cleavage by closing the N-terminal protein clamp (Ketron and Osheroff, 2014). Although cysteine 630 in human topoisomerase I is proximal to the active site tyrosine 723, the mechanism by which adduction increases the formation of DNA cleavage complexes by the type I enzyme is currently unknown.

The actions of camptothecin as a topoisomerase I poison have been known since the 1980s (Pommier, 2009). The work of Flor et al. demonstrates the complexity of translating enzymology to cellular pathways and emphasizes that we still have much to learn about the actions of camptothecins and the effects of stress on chemotherapy. It also suggests that it may be possible to modulate oxidative stress in order to exacerbate the actions of topoisomerase I-targeted drugs in cancer cells or mute them in non-cancerous tissues.

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DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

Ashley, R.E., and Osheroff, N. (2019). Recognition of DNA topology by topoisomerases: mathematics at the molecular level. In Knots, Low-Dimensional Topology and Applications, C.C. Adams, C.M. Gordon, V.F.R. Jones, L.H. Kauffman, S. Lambropoulou, K. Millet, J.H. Przytycki, R. Ricca, and R. Sazdanovic, eds. (Springer), pp. 411–433.

Deweese, J.E., and Osheroff, N. (2009). The DNA cleavage reaction of topoisomerase II: wolf in sheep's clothing. Nucleic Acids Res. *37*, 738–748.

Flor, A.C., Doshi, A.P., and Kron, S.J. (2016). Modulation of therapy-induced senescence by reactive lipid aldehydes. Cell Death Discov. *2*, 16045.

Flor, A.C., Wolfgeher, D., Wu, D., and Kron, S.J. (2017). A signature of enhanced lipid metabolism, lipid peroxidation and aldehyde stress in therapy-induced senescence. Cell Death Discov. *3*, 17075.

Flor, A., Wolfgeher, D., Li, J., Hanakahi, L.A., and Kron, S.J. (2020). Lipid-derived electrophiles mediate the effects of chemotherapeutic topoisomerase I poisons. Cell Chem. Biol. *28*, this issue, 776–787.e8.

Ketron, A.C., and Osheroff, N. (2014). Phytochemicals as anticancer and chemopreventive topoisomerase II poisons. Phytochem. Rev. *13*, 19–35.

Pommier, Y. (2009). DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition. Chem. Rev. *109*, 2894–2902.

Pommier, Y. (2013). Drugging topoisomerases: lessons and challenges. ACS Chem. Biol. *8*, 82–95.

Pommier, Y., and Osheroff, N. (2011). Topoisomerase-induced DNA damage. In DNA Topoisomerases and Cancer, Y. Pommier, ed. (Springer), pp. 145–154.

Pommier, Y., Sun, Y., Huang, S.N., and Nitiss, J.L. (2016). Roles of eukaryotic topoisomerases in transcription, replication and genomic stability. Nat. Rev. Mol. Cell Biol. *17*, 703–721.

Plug and play with recombinant antibody fragments

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In this issue of *Cell Chemical Biology*, Hentrich et al. (2021) describe the application of the SpyCatcher technology to antibody discovery and validation. Fab-SpyTag fusion proteins can be expressed in the periplasm of protease-deficient bacteria and coupled in a modular manner to a variety of SpyCatcher-tagged proteins for improved assay performance.

Various protein ligation techniques have been applied successfully to antibody engineering efforts. For example, enzymes such as sortase and subtilisin (Weeks and Wells, 2018, 2020) have been employed to ligate short peptides bearing biotin or fluorescent dyes to the N terminus or C terminus of recombinant antibody fragments, such as human single-chain fragments of variable regions (scFv) and Fabs. The ability to label antibodies away from their antigen

recognition sites ensures the efficient production of probes without the loss of functional binding. An alternative labeling strategy, involving the SpyTag/SpyCatcher system, was developed from the fibronectin binding protein, FbaB, from *Streptococcus pyogenes*, in which a 13 amino acid segment of the protein forms an isopeptide bond when it fits into a groove of a 140 amino acid domain (Zakeri et al., 2012) (Figure 1). This system has been used extensively in a variety of protein engineering applications (Keeble and Howarth, 2019).

The versatility of the SpyTag/SpyCatcher system has attracted interest for its application to the field of antibody engineering, with several early reports already published. In this issue of *Cell Chemical Biology*, the authors applied it to Fabs (Hentrich et al., 2021) and discovered that Fabs with SpyTag1 fused at the C terminus were subject to proteolysis when expressed in the periplasm of *E. coli* cells. (Antibody fragments are commonly expressed in the periplasm of bacteria due to the requirement of disulfide bond formation for proper folding, which is catalyzed by periplasmic disulfide isomerase.) When the heavy chain of a Fab was tagged with SpyTag1 and expressed in the periplasm, western blots revealed that the His-tag that was C-terminal to the SpyTag1 was missing, whereas the His-tag could be detected if it was N-terminal to the SpyTag1. This observation prompted the research team to investigate if tail-specific protease (Tsp) was causing loss of the SpyTag. While partial success was achieved with a cell strain in which the Tsp gene was knocked out, the group observed that Fabs tagged with SpyTag2 and SpyTag3, which differ in only a few amino acids from SpyTag1, were additionally truncated by proteases. As these two tags carry dibasic residues, which are known substrates of the outer membrane protease T (OmpT), the group created a double-knockout strain (lacking Tsp and OmpT activity) and demonstrated excellent expression of full-length SpyTag Fabs in the periplasm. With this experimental solution to the proteolysis problem in hand, the authors have gone on to express >1,000 different Fabs carrying C-terminal Flag-SpyTag2-His-tags. The average yield for the tagged Fabs expressed in the periplasm of Tsp-minus, OmpT-minus *E. coli* was 11 mg per liter culture.

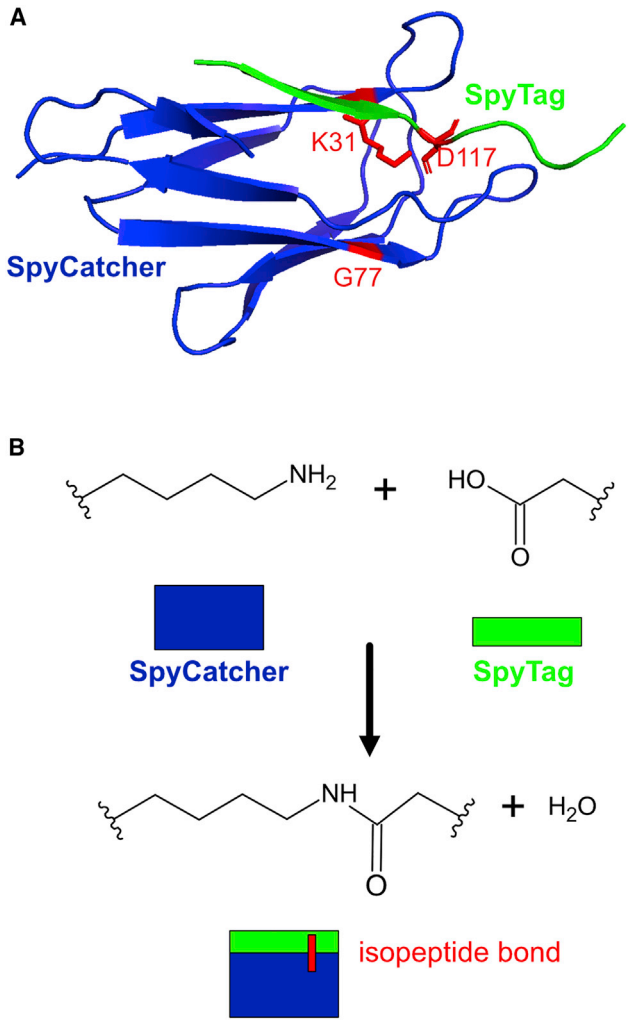

Figure 1. The SpyTag/SpyCatcher system

(A) Three-dimensional structure (PDB: 4MLI; Li et al., 2014) of SpyCatcher 1 (blue) complexed with SpyTag1 (green), with three key residues shown in stick form by PyMOL.

(B) Spontaneous formation of the isopeptide bond between the SpyTag and SpyCatcher (Keeble and Howarth, 2020). While SpyTag 1, 2, and 3 differ slightly in sequence and affinity for cognate SpyCatchers, they all form isopeptide bonds when complexed.

The authors then turned their attention to evaluating the SpyTag/SpyCatcher system for ligation of tagged Fabs to a variety of substrates (Figure 2). As anticipated, the Fab-SpyTag2 fusion proteins form covalent bonds with soluble SpyCatcher2 in 10 min, as evident by formation of larger molecular weight species by SDS-PAGE. Dimeric and oligomeric forms of SpyCatcher2 couple efficiently to Fab-SpyTag2 fusion proteins, although longer incubation times are required presumably due to the slight impact of steric hindrance. The authors additionally engineered forms of SpyCatcher2 with unpaired cysteines that allow directed chemical conjugation with horseradish peroxidase (HRP) or phycoerythrin (PE). Finally, the SpyTag2-Fab fusion proteins can be coupled to a fragment crystallizable (Fc) of immunoglobulin G (IgG), called an "FcCatcher," to generate a bivalent, IgG-like molecule.

These formats offer a number of benefits. First, by coupling Fabs to HRP- or PE-labeled SpyCatcher, secondary antibodies (i.e., goat anti-Fab-HRP, goat anti-Fab-PE) are not required and background signals are lower. Second, by coupling the Fabs to either dimeric SpyCatcher or SpyCatcher-Fc fusions, binding signals are increased due to the bivalent nature of the complex, which permits higher apparent affinities due to avidity. The authors illustrate these advantages in the improved assay performance of western blots, ELISA, flow cytometry, and immunofluorescence cell staining. This modular approach has the potential to streamline the process of characterizing and validating recombinant Fabs. Third, due to the exquisite and robust nature of the SpyTag/SpyCatcher interaction, it is possible to screen and evaluate crude lysates of hundreds of bacterial cultures in a high-throughput manner.

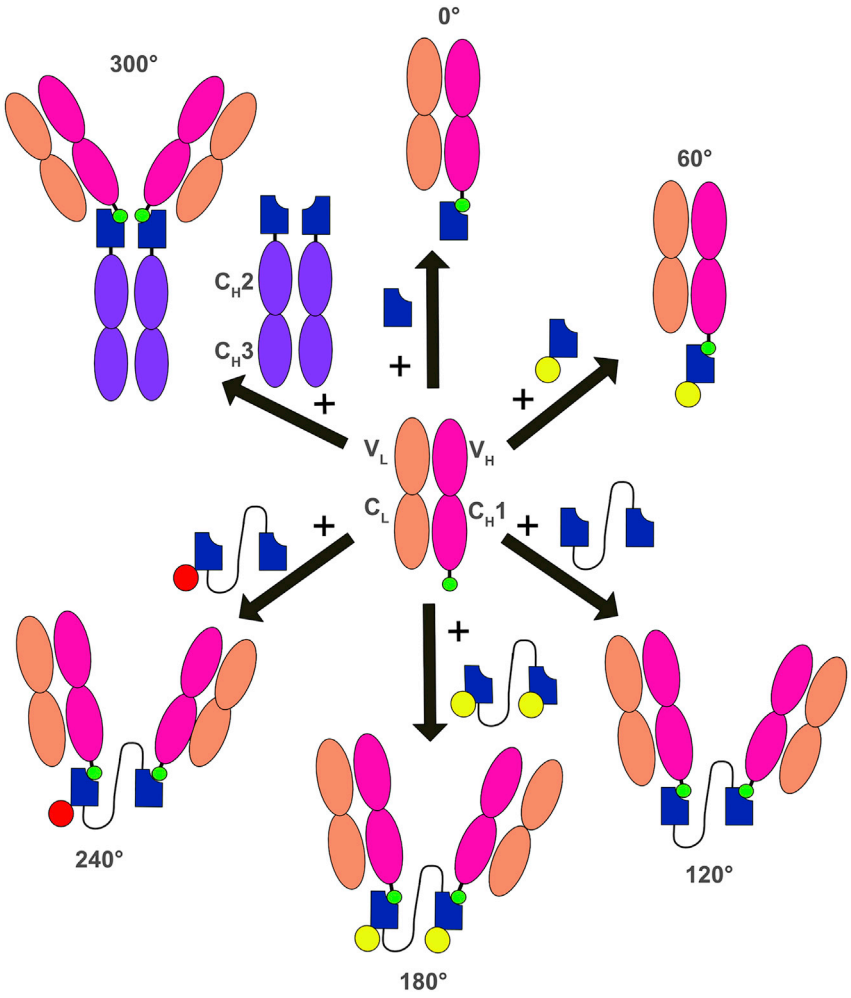

Figure 2. Modular assembly of Fabs into larger macromolecular structures

Fabs carry a SpyTag2 (green dot) at the C terminus of the heavy chain (magenta), which assembles with the light chain (salmon) in the periplasm of *E. coli.* Six different assemblies were successfully achieved. Radial representation: "0°," assembly of Fabs with SpyCatcher2 (blue); "60°," assembly of Fabs with SpyCatcher2 chemically conjugated to HRP (yellow); "120°," dimerization of Fabs with a tandem dimer of SpyCatcher2, separated by a linker (black line); "180°," dimerization of Fabs with a tandem dimer of SpyCatcher2 that has been conjugated to HRP; "240°," dimerization of Fabs with a tandem dimer of SpyCatcher2 that has been conjugated to PE; and "300°," dimerization of the Fabs with Fc fragments that carry SpyCatcher 2 at the N terminus of the C_H2 domain of heavy chain.

In conclusion, the authors convincingly establish the utility of the SpyTag/SpyCatcher system in the modular assembly of recombinant antibody fragments into labeled monovalent, bivalent, and oligomeric formats for diverse applications. This tour de force approach should accelerate the development of high-quality antibodies for research and diagnostic purposes. While the coupled proteins will not likely be considered for therapeutic use due to the immunogenic nature of SpyCatcher (a bacterial protein), the ability to generate IgG-like reagents easily *in vitro* should accelerate the selection of antibodies to move forward as possible therapeutic candidates.

DECLARATION OF INTERESTS

A.K.G. and C.J.M. are employees of Tango Biosciences. B.K.K. is a co-founder and shareholder of Tango Biosciences.

REFERENCES

Hentrich, C., Kellmann, S.J., Putyrski, M., Cavada, M., Hanuschka, H., Knappik, A., and Ylera, F. (2021). Periplasmic expression of SpyTagged antibody fragments enables rapid modular antibody assembly. Cell Chem. Biol. *28*, this issue, 813–824.e6.

Keeble, A.H., and Howarth, M. (2019). Insider information on successful covalent protein coupling with help from SpyBank. Methods Enzymol. *617*, 443–461.

Keeble, A.H., and Howarth, M. (2020). Power to the protein: enhancing and combining activities using the Spy toolbox. Chem. Sci. (Camb.) *11*, 7281–7291.

Li, L., Fierer, J.O., Rapoport, T.A., and Howarth, M. (2014). Structural analysis and optimization of the covalent association between SpyCatcher and a peptide Tag. J. Mol. Biol. *426*, 309–317.

Weeks, A.M., and Wells, J.A. (2018). Engineering peptide ligase specificity by proteomic identification of ligation sites. Nat. Chem. Biol. *14*, 50–57.

Weeks, A.M., and Wells, J.A. (2020). Subtiligase-catalyzed peptide ligation. Chem. Rev. *120*, 3127–3160.

Zakeri, B., Fierer, J.O., Celik, E., Chittock, E.C., Schwarz-Linek, U., Moy, V.T., and Howarth, M. (2012). Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. Proc. Natl. Acad. Sci. USA *109*, E690–E697.