biosynthesis pathway (which they named the futalosine pathway, after the first intermediate molecule). The lack of this pathway in humans and its presence in bacteria such as *Chlamydia* (which causes urethritis and respiratory tract infections), *H. pylori* (which can cause stomach ulcers), *C. jejuni* (which causes gastroenteritis often associated with food poisoning), and *Spirochaetes* (which cause syphilis and lyme disease) could make it an attractive antibacterial drug target for these specific pathogens.

The development pipeline for systemic antibiotics consists almost entirely of new versions of decades-old classes of antibiotics, such as β-lactams, quinolones, macrolides, and glycopeptides. New classes of antibacterial drugs directed against new bacterial targets are urgently needed. Unfortunately, there are insufficient novel antibiotics in development to address this challenge, partly because of decreased investment in this sector and also because of the substantial difficulty of finding small-molecule drug leads. Despite a wealth of new bacterial targets, high-throughput screening for inhibitory compounds in this therapeutic area has been less successful than in any other (*8*), a likely cause being the unique chemical diversity needed to inhibit bacterial enzymes. Consequently, the work by Haydon *et al.* and Rasko *et al.* is important because they have identified inhibitors of their targets with the potential for pharmaceutical development. However, turning these "leads" into drugs remains a challenge. For example, antibacterials typically need to be administered at higher doses than most other drugs, emphasizing the need for compounds that can achieve high exposures in humans but that are also extremely safe at these high doses

For the last 10 to 15 years, antibacterial research and development has focused on the validated approach of designing small molecules that inhibit bacterial growth. However, perhaps now is the time to consider alternative strategies. For example, targeting virulence factors as described by Rasko *et al.* may create more effective drugs with a lower propensity to select for resistance. However, the amount of attenuation achieved by such antivirulence drugs and the consequences of potentially not eradicating the bacteria from the infection need careful consideration. Such drugs may need to be combined with antibacterial agents to achieve their full potential. Other promising approaches include developing inhibitors of bacterial drug resistance mechanisms or bacterial drug efflux pumps for combination with specific antibacterials that could rejuvenate entire classes of antibiotics against multidrug-resistant pathogens. In addition, rather than the traditional approach of seeking antibiotics that cover a broad set of pathogens, exploiting targets that are specific for only certain pathogens, such as those described by Hiratsuka *et al.*, may be a more productive strategy. This would also have the advantage of creating antibiotics that will enable highly targeted therapy and remove the considerable drug discovery challenge of having to identify a single molecule that penetrates, and is equipotent against, a range of potentially diverse species of bacteria (*8*). However, this approach will succeed only be with the availability of diagnostics that can very rapidly and accurately identify the specific infecting pathogen, and it may be some time before such tools are available for a range of common pathogens. Consequently, the need for new antibiotics merits investment across a spectrum of traditional and higher-risk approaches to optimize the chances of creating promising new antibiotics.

References

1. G. Taubes, *Science* **321**, 356 (2008).
2. R. M. Klevens, M. A. Morrison, S. K. Fridkin, *JAMA* **17**, 1763 (2007).
3. The Royal Society, "Innovative mechanisms for tackling antibiotic resistance"; http://royalsociety.org/displaypagedoc.asp?id=30717 (2008).
4. B. Spellberg *et al.*, *Clin. Infect. Dis.* **46**, 155 (2008).
5. D. J. Haydon *et al.*, *Science* **321**, 1673 (2008).
6. D. A. Rasko *et al.*, *Science* **321**, 1078 (2008).
7. T. Hiratsuka *et al.*, *Science* **321**, 1670 (2008).
8. D. J. Payne, M. N. Gwynn, D. J. Holmes, D. L. Pompliano, *Nat. Rev. Drug Discov.* **6**, 29 (2007).

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CHEMISTRY

Fluorous Tags Unstick Messy Chemical Biology Problems

Separation and identification of biological molecules from complex mixtures can be made easier with fluorinated labeling groups and separation media.

Dennis P. Curran

Everyone knows that nothing sticks to Teflon-coated products, such as cookware, raincoats, and ski waxes (and, figuratively, even to some politicians). The prevalence of "nonstick" products coated with Teflon [poly(tetrafluoroethylene)] shows that with some engineering effort, Teflon can adhere to metals, textiles, and plastics. At the molecular level, the perfluoroalkyl groups [$–(CF_2)_n–$] that comprise Teflon tend to repel organic and inorganic molecules but have attractive interactions with other perfluoroalkyl (R_F) groups and along with fluorinated solvents can form separate fluorous phases. Organic chemists exploit perfluoroalkyl groups in small-molecule synthesis and separation by applying them as tags for separations with fluorous silica gel and solvents. Recent innovations suggest that a wide range of potential applications of fluorous tags could be realized in chemical biology as well, not only in separations and derivatization but also in identification because of the distinctive signatures of these tags in mass spectrometry.

Separation tags can enable rapid partitioning of a relatively complex mixture (such as cell isolates or products of cell-based protein synthesis) into tagged and untagged fractions. For example, a streptavidin affinity column will fasten molecules with a biotin tag and let the untagged molecules pass. Separation tags that are commonly used with biomolecules include polymer beads or surfaces, as well as other molecular tags such as polyhistidine.

Given the success of these commonly used separation tags, why are fluorous tags of interest? First, separation tags typically also have to accommodate—better yet, facilitate—biomolecule synthesis and analysis methods. Fluorous tags provide separation handles that are relatively inert and do not compromise synthetic reactions or analysis operations.

Second, tag systems such as streptavidin-biotin rely on very strong fastening interactions (covalent bonds or powerful ionic or molecular recognition forces) that may be difficult to unfasten during product recovery. Fluorous tags behave more like molecular "Post-it notes." For example, when synthetic chemists use fluorous solid-phase extraction

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(FSPE) for separations (*1*), only the fluorous-tagged molecules stick to the gel column in the first elution (typically in a wet organic solvent). The tagged molecules are then easily washed off and captured with a fluorophilic solvent. In a very early chemical biology application, a group at Dupont reacted the free amines of proteins with fluorous isocyanates ($R_FN=C=O$) and showed that the resulting fluorous-tagged proteins could be absorbed onto both solid and liquid fluorous phases (*2*).

Fluorous separation techniques have an added bonus if the subsequent identification steps involve mass spectrometry (MS). In fluorous proteomics (see the figure, top panel), a complex peptide (or protein) sample is subjected to a selective reaction with a fluorous reagent that targets a specific chemical functional group, such as thiols of cysteines (*3*). The resulting complex sample of labeled and unlabeled peptides is then dramatically enriched in the labeled peptides by FSPE. Subsequent MS analysis to identify the fluorous-tagged peptides and, hence, their precursor proteins is actually easier than with untagged proteins. The fluorous tags are not prone to fragmentation, so the spectra are less complicated. Furthermore, molecules bearing fluorous tags are easily ionized and have mass peaks that are readily identified. The fluorous reagents and separation materials are also inexpensive compared to those used in established proteomics techniques.

Fluorous methods are also proving useful in the synthesis and microarray analysis of polysaccharides and sugar derivatives, which are much more challenging than proteins (see the figure, middle panel). Monosaccharides bearing a fluorous tag at the anomeric center (the carbon atom that determines the stereochemistry of the glycoside linkage) have been converted to disaccharides by using the techniques of fluorous synthesis (*4*). Here, standard methods of solution-phase carbohydrate synthesis are used, but the fluorous tags enable separation of the target products from by-products much more rapidly than in other approaches.

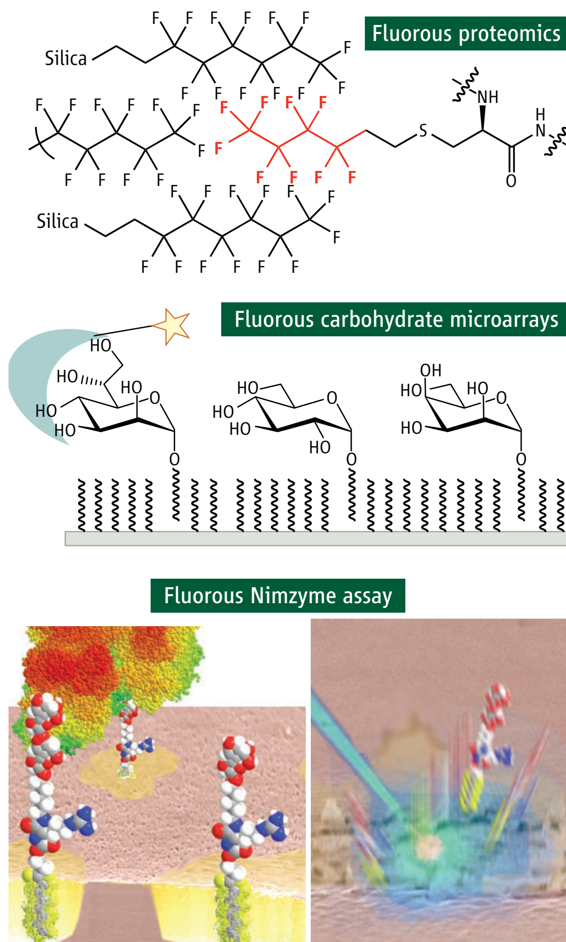
Separation tags are often removed at the end of such syntheses, much like removing packaging materials from a shipment. Microarray methods usually involve a "repackaging" of the molecules so that they can be attached by covalent bonds onto the slide. Another bonus of the fluorous approach is that there is no repackaging; the fluorous-tagged carbohydrates can be directly spotted onto fluorous slides to make microarrays for screening.

Like fluorous silica gel, the slides have a fluorous bonded phase, and the affinity of the tags for the bonded phase allows the slides to be washed with detergent solutions during analysis without removing the spots or compromising their morphologies. The spotted slides are exposed to fluorescently labeled lectins (selective carbohydrate-binding proteins) for analysis. The nonstick nature of the fluorous slides minimizes nonspecific adsorption of lectins other than to the carbohydrate target. The fluorous-fluorous interaction suffices to hold tagged molecules onto the slide, but like Post-it notes, the fluorous spots can easily be peeled off after analysis. This technique has already yielded information on binding of heptoses to lectins (*5*) and identified new small molecule inhibitors of histone deacetylase (*6*).

The recently introduced technique of nanostructure-initiator mass spectrometry (NIMS) is showing promise as an alternative to current MS analysis methods (*7*) for large biomolecules that are not readily volatilized. In the original incarnation of NIMS, perfluorosiloxanes were used as initiators for laser vaporization of analytes into the gas phase. The fluorous features of these initiators have also been captured and used in an enzymatic assay (see figure, bottom panel). In proof-of-principle experiments, a fluorous-labeled disaccharide was immobilized in the fluorous nanopores of a NIMS chip. The chip was exposed to enzymes that either add or remove a saccharide ring. Subsequent MS analysis directly detected the fluorous-tagged elongated or truncated products. The sensitivity of this so-called Nimzyme assay for the presence of enzymes in solution was much better than traditional colorimetric assays and was comparable with fluorescence methods (500-fg level).

The Nimzyme analysis has already been used to characterize the enzymatic activity of crude cell lysates from thermophilic bacteria. Here, the ability to wash away the huge background of the cell lysate while retaining the fluorous-tagged enzyme products for MS analysis is central to the success of the assay. That, combined with the inherent attractive features of NIMS, recommends the assay for high-throughput bioprospecting applications.

These early innovations herald the expansion of fluorous methods into chemical biology. If they catch hold, then perhaps we can say that something sticks to Teflon after all.



Just sticky enough. (**Top**) Fluorous proteomics. A polypeptide bearing a fluorous tag on a cysteine is held onto a fluorous silica gel column during a first elution while nontagged molecules pass through. (**Middle**) Carbohydrate microarrays. An assortment of fluorous-tagged carbohydrates are arrayed into spots on a slide having a fluorous bonded phase. A fluorescently labeled lectin (blue region with star) binds to the carbohydrates but washes readily off the fluorous slide. (**Bottom**) Nimyzme assay (*7*). A glycosidase enzyme in a crude lysate operates on a substrate held to a surface by a fluorous siloxane. The lysate is washed away. The siloxane now functions as an initiator to help quantify the activity by MS analysis. [Copyright (2008) National Academy of Sciences USA]

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References

1. W. Zhang *et al.*, *Tetrahedron* **62**, 11837 (2006).
2. R. K. Kobos *et al.*, *Tibtech* **7**, 101 (1989).
3. S. M. Brittain *et al.*, *Nat. Biotechnol.* **23**, 463 (2005).
4. K. S. Ko *et al.*, *J. Am. Chem. Soc.* **127**, 13162 (2005).
5. F. A. Jaipuri *et al.*, *Angew. Chem. Int. Ed.* **47**, 1707 (2008).
6. A. J. Vegas *et al.*, *Angew. Chem. Int. Ed.* **46**, 7960 (2007).
7. T. R. Northen *et al.*, *Proc. Nat. Acad. Sci. U.S.A.* **105**, 3678 (2008).

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