all of the effects of long-term inhibition of this enzyme in people with diabetes.

In order to tackle this problem, Tagore *et al.*[3] made use of liquid chromatography followed by mass spectrometry to monitor both the relative abundance and the identity of all small peptide fragments in samples obtained from both normal mice and mice that had been either genetically engineered or chemically treated to lose DPP4 activity (**Fig. 1**). Using this comparative approach, the authors were able to focus in on only those fragments that were either elevated or diminished upon depletion of DPP4 activity. This process of profiling changes in total metabolites, often referred to as metabolomics, resulted in the identification of a number of putative substrates of DPP4, including some that were previously unknown. In addition, because the mass spectrometry method allowed each of these peptides to be molecularly characterized, the authors were able to define the exact sites where a cut occurred. Their results indicated that, in addition to the processing of GLP-1, DPP4 also acts on a number of other substrates. Furthermore, the authors demonstrate that some of the newly identified peptides are further trimmed by the action of other peptidases to produce additional downstream metabolites.

Overall, this study reveals new insights into how DPP4 functions and provides a better understanding of the potential effects (beyond those on GLP-1 signaling) of DPP4 inhibition.Though the list of new substrates of DPP4 produced by this study is likely not comprehensive, it serves as a starting point for future studies to validate new leads in order to get a clearer picture of the protease networks that are controlled by DPP4.

Metabolomics is a rapidly growing field of study that has traditionally focused on mapping small-molecule metabolites. As we become aware of an increasing number of examples of peptide metabolites that regulate key biological processes, we will need to further direct our attention toward analytical methods that allow pools of peptide metabolites to be monitored. The study in this issue provides a critical step forward in the development and application of such a method.

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Engineering fluorination

Graham Sandford

Cytochrome P450 enzymes selectively oxidize relatively unactivated sites in a range of model drug-like substrates *in vitro*. The hydroxylated products can be transformed into selectively fluorinated systems, providing a rapid sequential method for the identification, activation and fluorination of saturated sites in drug candidates.

Many selectively fluorinated molecules have very valuable biological activity, and indeed some of the most commercially successful pharmaceuticals on the market today, such as fluoxetine (Prozac) and ciprofloxacin, exemplify the importance of fluorinated compounds to the clinic[1]. Fluorination of biologically active systems as part of hit-to-lead medicinal chemistry campaigns can provide new chemical entities that have, for example, enhanced membrane permeability, protein binding, biologically compatible pK_as and enhanced metabolic stability[2,3]. Consequently, many fluorinating agents capable of transforming appropriate functionality into CF, CF_2 and CF_3 groups have been developed to meet the demands of life science discovery chemists. Selective fluorination strategies usually involve either functional group transformation of suitable leaving groups by fluoride ions or reaction of unsaturated systems, such as enolate or aromatic derivatives, with an electrophilic fluorinating agent[1]. Fluorination of carbon sites that are adjacent to functionality is therefore possible in many cases, but methodologies that allow efficient regioselective and stereoselective late-stage fluorination of complex molecular scaffolds at sites that are remote from any functionalization have not been developed to any great extent[4]. Rentmeister *et al.* have now developed an enzyme-based method for the selective fluorination of sites remote from functionality in a range of biologically active systems that aims to address this requirement[5].

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A crucial task in the development of new pharmaceutical products is the determination of the metabolic fate of a drug candidate in the human body and the subsequent impact of potentially toxic metabolites on human health[6]. The strategy of blocking metabolically vulnerable sites of lead compounds in drug discovery programs by the replacement of hydrogen by fluorine at positions particularly sensitive to oxidation has been highly successful in the past[2,3]. For example, in the cholesterol absorption inhibitor ezetimibe, fluorination and other structural modifications reduced metabolic degradation to such levels that the required dose for activity could be decreased by 55 times while increasing activity 400-fold (**Fig. 1a**)[2].

Rentmeister *et al.*[5] have now successfully exploited techniques that apply both (i) *in vitro* oxidation of unactivated, readily oxidized, unfunctionalized sites in drug candidates by mutant cytochrome P450 enzymes and (ii) subsequent fluorination of the resulting hydroxyl functionality that arises from chemo-enzymatic transformation (**Fig. 1b**). Initially, model substrates based on the cyclopentenone system, a scaffold present in many natural products such as jasmonoid, cyclopentenoid antibiotic and prostaglandin derivatives, were subjected to enzymatic oxidation by variants of the bacterial long-chain fatty acid hydroxylase $P450_{BM3}$ from *Bacillis megaterium*. Though regioselective and stereoselective chemical transformations of cyclopentenoid systems can be very difficult, 30 to 50% of the enzyme variants led to high levels of substrate oxidation with very useful levels of selectivity. Upon identification of the most active enzyme variants, chemo-enzymatic reactions were scaled up to preparative procedures, and subsequent transformation of the resulting

hydroxylated sites by the well-established fluorinating reagent diethylaminosulfur trifluoride (DAST) successfully furnished the corresponding monofluorinated systems.

Next, the tandem oxidative fluorination strategy was applied to the synthesis of fluorinated derivatives of commercial pharmaceutical substrates, particularly the methylester pro-drug of ibuprofen, the highly effective and generally administered anti-inflammatory agent. Fluorination of unactivated remote saturated sites of the pendant *iso*-butyl group was possible using the approach described by Rentmeister *et al.* (**Fig. 1c**). After isolation, the resulting fluorinated systems were found to have substantially improved membrane permeability over the parent ibuprofen pro-drug derivative, and they also had (of course) greater metabolic stability because the most readily oxidized site had been 'blocked' by selective fluorination.

The chemo-enzymatic approach of Rentmeister *et al.* allows, in many cases, fluorination of relatively unreactive saturated sites in a variety of organic systems in preparatively significant yields for the discovery chemist, thereby greatly extending the opportunities for the synthesis of selectively fluorinated bioactive systems. Developments in the scale-up of the chemo-enzyme approach to provide multigram quantities of fluorinated materials and improvements in the stereoselectivity of the fluorination stage will undoubtedly grow out of this research, providing the medicinal chemist with new ranges of fluorinated chemical entities and leading to a wider appreciation of the role played by fluorine in medicinal chemistry.

Rapid identification of the most readily oxidized positions of drug candidates, subsequent functionalization by oxidation, and finally 'blocking' of the sites by fluorination to provide fluorinated chemical entities with improved *in vivo* metabolic stability and increased lipophilicity (depending upon the reaction site) is potentially a very useful complimentary feature of the Rentmeister *et al.* chemo-enzymatic oxidation-fluorination strategy. This simple two-step concept could find very wide application in hit-to-lead drug discovery programs where early identification of metabolically sensitive sites, bioassays and appropriate structural modification of drug candidates to more oxidatively stable, less toxic lead fluorinated compounds that may have enhanced biological activity could be rapidly achieved with minimal resources. Given that 20% of all pharmaceuticals and 30% of plant protection agents are fluoroorganic systems[3], new effective, selective fluorination methodologies for unactivated sites may generate many novel classes of fluorinated systems with increased bioactivity.

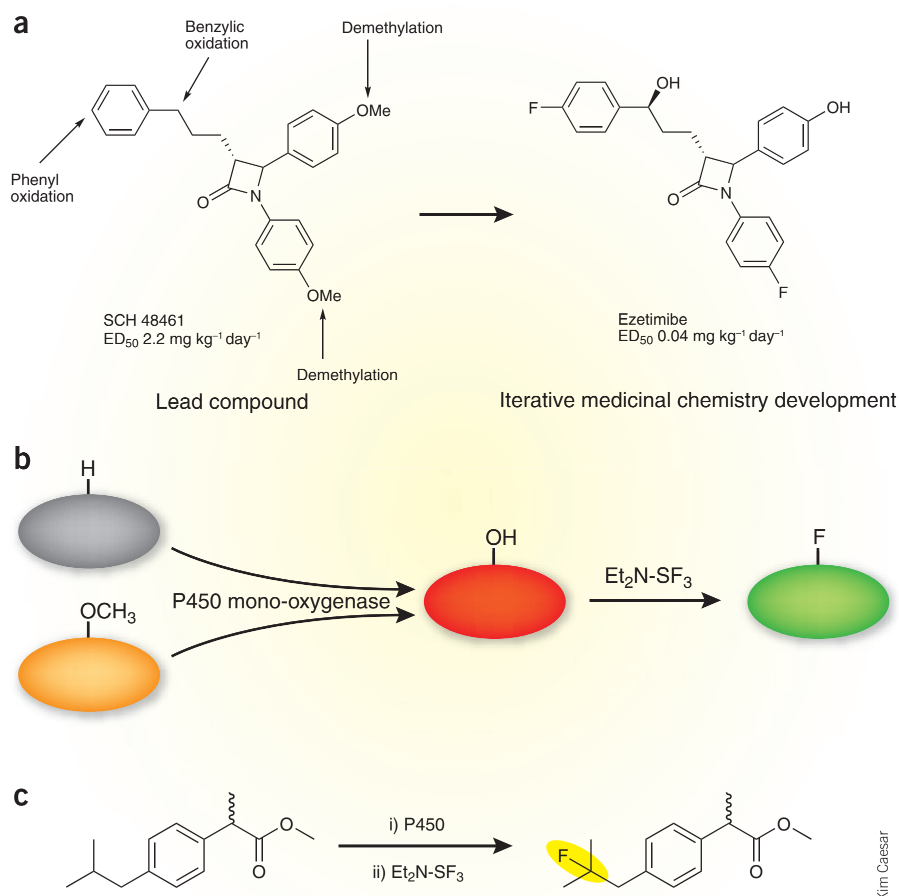

Figure 1 Engineering fluorination. (**a**) Fluorinated pharmaceuticals. Development of lead compound SCH 48461 into ezetimibe, a more potent and metabolically stable agent that inhibits cholesterol reabsorption. (**b**) General chemo-enzymatic strategy for fluorination of unactivated sites in organic substrates. (**c**) Chemo-enzymatic fluorination of an ibuprofen derivative.

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