# Metabolomics cuts to the chase to chase the cuts

Matthew Bogyo

**Peptidases are enzymes that trim small protein fragments called peptides to regulate their biological functions. A new method opens the door to chasing down and identifying important cutting events mediated by peptidases involved in metabolic regulation.**



Proteases are enzymes that perform the basic task of cutting up other proteins and peptides by hydrolysis of amide bonds. In most organisms, proteases make up about 2% of the genome[1] and can be grouped into subfamilies based on how and where they carry out cutting of a substrate[2]. The alternative name, peptidase, is often used to describe proteases that act on small peptide substrates rather than proteins. Though proteases and peptidases were once thought to be the uninteresting "garbage disposal units" of the cell, it is now clear that specific and partial proteolysis of protein and peptide substrates plays a critical role in regulating the function of a large and diverse array of signaling molecules. The process of cutting a protein or peptide into smaller pieces can lead to either a gain or loss of function of the substrate. In addition, most proteases act on more than one substrate, resulting in a complex array of possible outcomes that can be controlled by a single protease. Thus, methods that can be used to globally define all the proteolytic processing events mediated by a given protease are often required to fully understand its true range of biological functions. The need to get such a global picture of proteolytic pathways is heightened in cases where a protease is a target for drugs designed to treat human diseases. Having a complete picture of these regulatory networks is critical to our ability to predict the behavior of a drug when used to treat people.

In this issue of *Nature Chemical Biology*, Tagore *et al.* profile the levels of peptide metabolites (the 'metabolome') that are produced by dipeptidyl peptidase 4 (DPP4)[3], which removes the first two amino acids (dipeptides) at the N terminus of a peptide substrate. One of its key metabolic regulatory roles is to cleave the peptide hormone glucagon-like peptide-1 (GLP-1), which in turn leads to a reduction in the secretion of insulin by the beta cells of the pancreas[4]. The identification of this critical metabolic function has prompted the development of a number of small-molecule inhibitors of DPP4, including sitagliptin (Januvia), which was recently approved by the US Food and Drug Administration for the treatment of type II diabetes[5]. However, like most proteases, a complete picture of the targets of DPP4 is lacking, which makes it difficult to anticipate

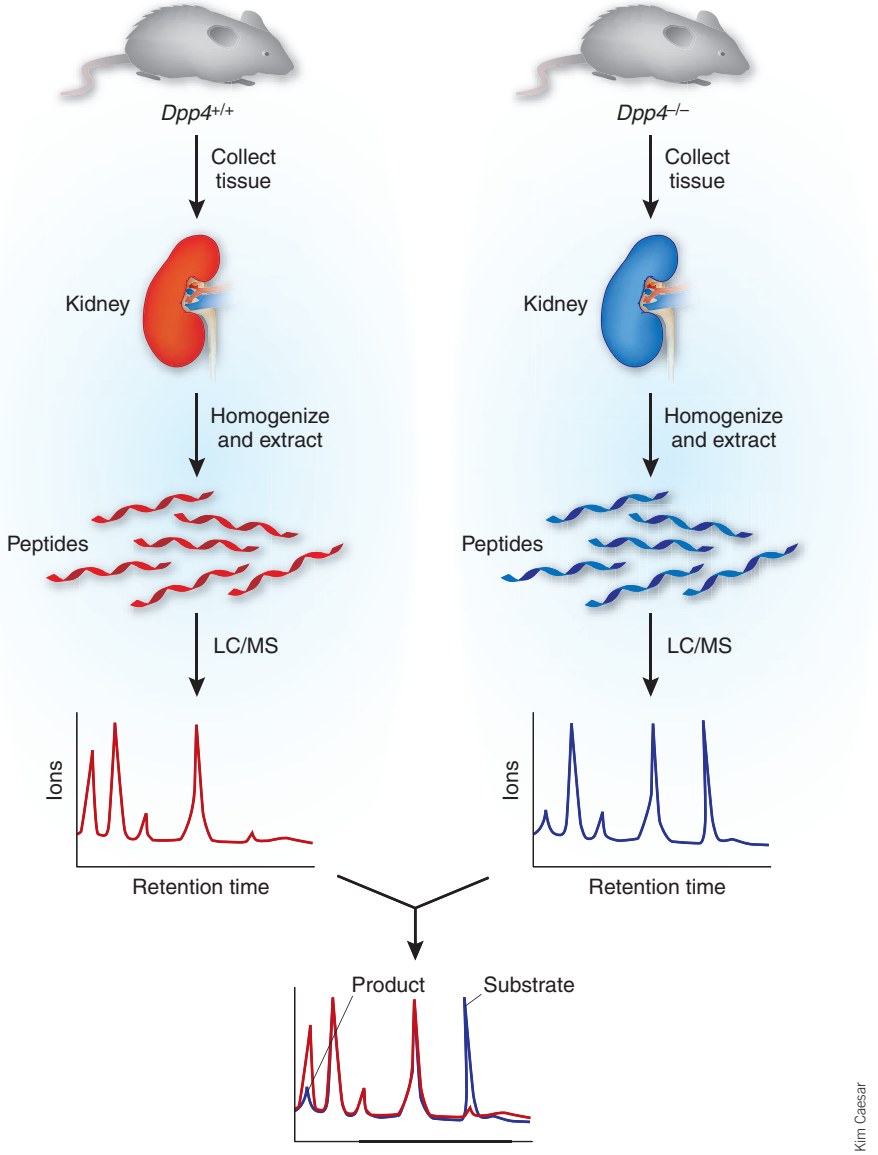


**Figure 1** Metabolomic identification of downstream substrates of DPP4. Kidneys from wild-type (*Dpp4*$^{+/+}$) and DPP4-deficient (*Dpp4*$^{-/-}$) mice are homogenized and peptides are isolated by extraction. The total pool of peptides is then analyzed by LC/MS. This method allows both the identity and the relative abundance of each peptide to be measured for each sample. By direct overlay of the LC/MS profiles, it is possible to identify both substrates of DPP4 (for example, peptides that increase in the *Dpp4*$^{-/-}$ sample) and products produced by DPP4 action (for example, peptides that decrease in the *Dpp4*$^{-/-}$ sample).

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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

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**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 p$K_a$s 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].

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

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