however, its spatial resolution and sensitivity are limited. Numerous matrix-assisted laser desorption/ionization (MALDI) MS imaging studies have reported biomarker identification for prognosis of lung and brain cancers, and also for elucidating disease mechanisms (McDonnell et al., 2010). In cytoskeleton research, MALDI imaging has been used to locate the interaction of unlabeled small molecules with microtubules.

In conclusion, studies that examine effects of small molecules and aim to identify their specific cellular targets need to include specific cellular readouts at the molecular level together with a complementary set of in vivo and in vitro techniques for their unambiguous validation, thus focusing basic and clinical research on the right targets and avoiding erroneous conclusions and unfounded claims.

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Activity-Based Profiling of 2-Oxoglutarate Oxygenases

Paul P. Geurink,[1] Bogdan I. Florea,[2] and Herman S. Overkleeft[2,*]
[1]Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
[2]Leiden Institute of Chemistry and the Netherlands Proteomics Centre, Einsteinweg 55, 2300 RA Leiden, The Netherlands
*Correspondence: h.s.overkleeft@chem.leidenuniv.nl
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2-Oxoglutarate oxygenases (2-OGs) are a large enzyme family involved in numerous processes in health and disease. Rotili et al. (2011) describe in this issue of *Chemistry & Biology* an activity-based protein profiling-based strategy with which the activity of individual members of the 2-OG family can be addressed in the context of complex biological systems.

Activity-based protein profiling (ABPP) has emerged as a powerful strategy in chemical biology research involving enzymes (Evans and Cravatt, 2006). In ABPP research, activity-based probes (ABPs) are designed to recognize, and subsequently bind covalently to, an enzyme or enzyme family, preferably in complex biological systems. An ABP is normally assembled from three individual functional parts: a recognition element (generally but not necessarily derived from the substrate of the enzyme at hand), a reactive group (for instance, electrophilic trap, photoreactive group), and an affinity tag. ABPP studies on enzymes are complementary to classical enzyme inhibition studies, in which an isolated enzyme reacts with a fluorogenic substrate, either in the presence or absence of a competitive inhibitor. Inhibition constants can be accurately measured in this fashion, which provides valuable information in case the inhibitor at hand is considered as a lead for drug development.

The kinetics studies of covalent, irreversible inhibitors are considerably more complicated. For this reason, and perhaps more importantly also for the widespread belief in medicinal chemistry that such compounds would make poor drug candidates, covalent inhibitors have been neglected for some time. This situation changed drastically a decade ago with a number of seminal studies. Biotinylated broad-spectrum inhibitors of serine hydrolases (Liu et al., 1999) and cysteine proteases (Greenbaum et al., 2000) proved highly useful in the profiling of these hydrolytic enzymes in cell extracts and living cells. At the same time, biotinylation of the natural product epoxomicin led to the identification of proteasomes as the target of this toxic, but potentially therapeutic, agent (Meng et al., 1999). These groundbreaking studies have opened the field of ABPP, and several attractive aspects of the strategy have been addressed in the following years. As said, transforming a natural product into an ABP may reveal its biomolecular target. Enzyme activities may be unearthed, or the presence (or absence) of

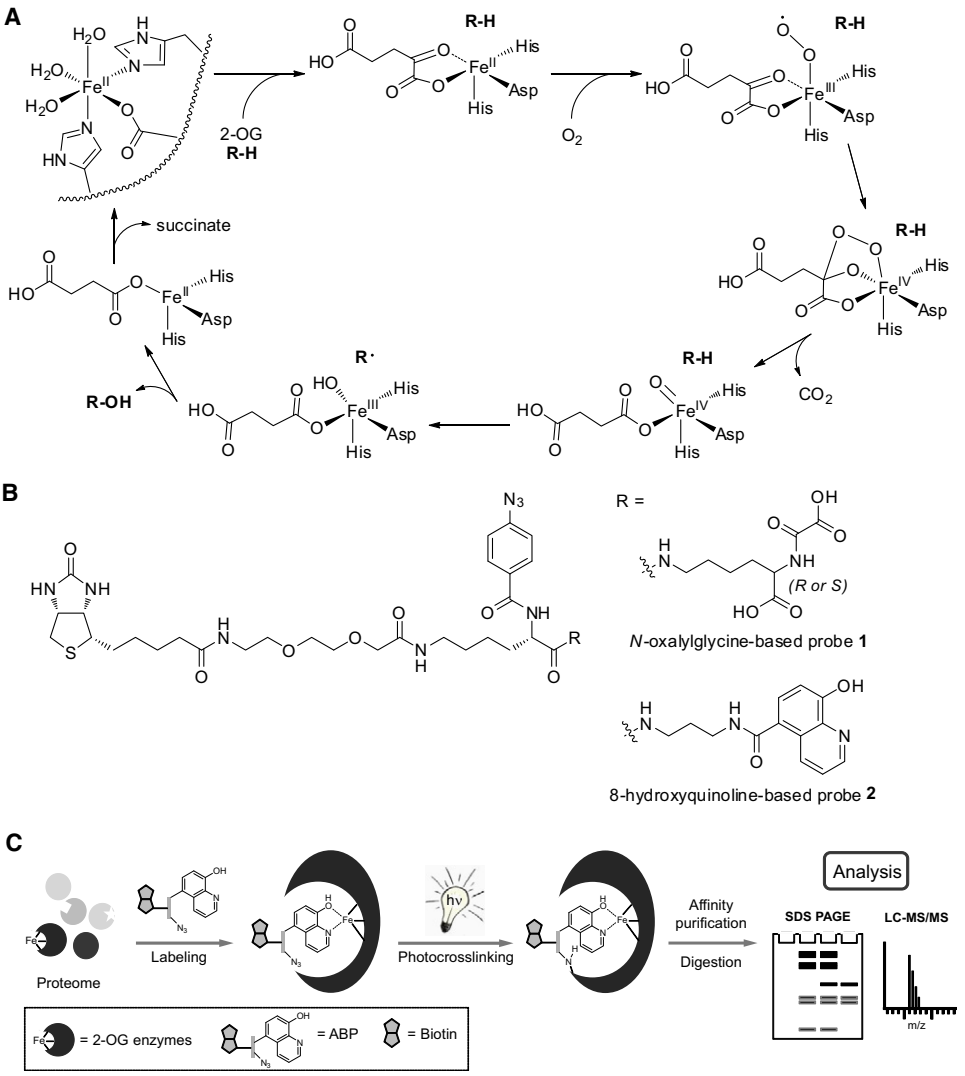

Figure 1. Rational Design of Activity-Based 2-OG Probes
(A) General mechanism of 2-oxoglutarate-dependent oxygenases.
(B) Activity-based 2-OG probes employed in the highlighted studies.
(C) General flowscheme of activity-based 2-OG profiling.

a given enzymatic activity in a biological system may be demonstrated in a chemical proteomics setting. ABPs provide the means to perform competition studies in biological systems, that can reveal the selectivity (or lack thereof) of a given inhibitor. Last, ABPs derived from putative substrates will tell whether these are viable enzyme substrates.

Obviously the success of ABPP research relies on whether suitable ABPs for the target enzyme, or enzymes, are within reach. Not surprisingly, most early successes entailed studies involving enzymes that employ an active site nucleophile (for instance, serine, cysteine, threonine) for which electrophilic traps (fluorophosphonate, epoxysuccinate, epoxyketone) were already known, more often than not, as part of natural products. Serine/threonine/cysteine hydrolases are often abundant and are often somewhat lenient with respect to their substrates, allowing installation of an affinity tag. However, many other enzyme families exist that would benefit from ABPP strategies provided that suitable ABPs are available. Recent years have witnessed progress in bringing such enzymes into the ABPP arena, including glycosidases (Witte et al., 2010), matrix metalloproteases (Saghatelian et al., 2004) and kinases (Hagenstein et al., 2004). A specific feature of the latter two, which do not employ an active site nucleophile embedded in the polypeptide backbone, is the inclusion of photoreactive groups into the ABP. This feature is also the distinctive factor in the work described in this issue (Rotili et al., 2011), in which Fe(II)/α-ketoglutarate-dependent oxygenases are subjected to ABPP studies. These enzymes, also known as 2-oxoglutarate oxygenases (2-OG, see Figure 1A for a general mechanism), are responsible for a wide number of chemical transformations throughout the kingdoms of life (Hausinger, 2004).

In humans, some 60 enzymes are thought to belong to this class including proline/lysine hydroxylases, but also histone demethylases. The physiological roles they exert range from gene control (DNA repair, DNA modifications, regulation of transcription factors) over oxygen sensing to metabolic processes. From an organic chemical point of view, the nature of the transformations achieved by 2-OG; that is, regio- and stereoselective manipulation of unactivated C-H bonds is highly attractive. This holds true also particularly for microorganismal 2-OG enzymes, such as the penicillin synthases and halogenases.

Kessler and Schofield and colleagues reveal in this issue and for the first time the design and application of 2-OG probes in biological systems (Rotili et al., 2011). They explored two ABPs (Figure 1B), one (compound **1**) based on the 2-oxoglutarate analog oxalylglycine and the other (compound **2**) on the known 2-OG inhibitor 8-hydroxyquinoline. Both compounds are equipped with a phenyl azide as the photoreactive group and a biotin for identification purposes. Of the two probes, the oxalylglycine derivative proved unsuitable for use in ABPP. The hydroxyquinoline derivative, however, met with more success. In a key experiment (Figure 1C), they treated nuclear protein extracts with the ABP compound **2**, followed by streptavidin pulldown and SDS PAGE analysis. One of the resulting bands proved to correspond to the histone demethylase FBXL11. Compound **2** is a first-of-its-kind ABP, with which 2-OG proteins can be identified and enriched from complex mixtures using ABPP. In a series of related experiments, the authors demonstrate that hypoxia-related 2-OGs can also be enriched, which led them to show that expression levels of the target 2-OGs is dependent on the oxygen levels. Functional chemical biology studies involving activity-based 2-OG profiling are now realistic.

Obviously, there is room for improvement. As the authors state, the hydroxyquinoline is not likely to become the reagent of choice for broad-spectrum ABPP of 2-OGs. Somewhat surprisingly, the oxalylglycine derivative failed to give the desired result, but the attachment of the affinity label and biotin might have added considerable bulk to the 2-oxoglutarate scaffold. Possibly, and also noted by the authors, two-step bioorthogonal labeling may come to the rescue here (Ovaa et al., 2003). Overall, the paper not only adds to the growing list of enzymes amenable for ABPP studies, but also sets the stage for future research, both in the development of improved 2-OG ABPs and in applying these in physiological studies.

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Methylations: A Radical Mechanism

Marc Fontecave[1,2,*]
[1]Institut de Recherches en Technologie et Sciences pour le Vivant IRTSV-LCBM, UMR 5249 CEA/CNRS/UJF, CEA-Grenoble, 17 avenue des Martyrs, 38054, Grenoble Cedex 09, France
[2]Collège de France, 11 place Marcellin-Berthelot, 75005 Paris, France
*Correspondence: mfontecave@cea.fr
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On the basis of labeling experiments, Grove et al. (2011) have shown how an electrophilic carbon (from an RNA adenosine) can be methylated by S-adenosylmethionine-dependent methyltransferases though an original radical mechanism.

The diversity of proteins and nucleic acids rests on the combination of a very limited number of chemical bricks, 20 amino acids and 4 nucleotides, respectively. Even though this leads to a huge number of combinations, in order to extend this diversity further living organisms have evolved additional selective mechanisms which allow the direct and site-specific incorporation of chemical groups into these macromolecules (Walsh, 2006; Grosjean, 2009). These modifications