

Proteomes take the electrophilic bait

Thomas Kodadek

New electrophilic reagents for activity-based protein profiling (ABPP) can discriminate between nucleophilic amino acids, such as cysteine, that are in different protein microenvironments. These reagents extend the reach of ABPP beyond serine proteases and other proteins with hyperreactive nucleophiles.

Proteomics researchers are constantly on the lookout for new and better ways to fractionate their samples since the mind-numbing complexity of virtually any proteome easily defeats the ability of even the most advanced mass spectrometers or other instruments to “see” all of the molecules in the sample. In this issue of *Nature Chemical Biology*, Weerapana *et al.* report new chemical tools for this purpose¹. They demonstrate that particular electrophilic molecules that one might imagine would have similar reactivities in fact display largely nonoverlapping labeling of proteins in complex mixtures. These reagents thus constitute a set of chemical ‘baits’ to catch different kinds of protein ‘fish’.

Most large-scale proteomics projects, for example those focused on biomarker discovery in serum or tissue homogenates or other interesting biological samples, use chromatographic fractional upfront to simplify the samples². An alternative idea is to separate proteins based on their differential chemical reactivity instead of how tightly they bind to a chromatography column. This approach has been called activity-based protein profiling (ABPP)³. Initially, ABPP studies used molecules that were essentially suicide inhibitors of particular classes of enzymes, for example the reaction of fluorophosphonates with serine peptidases, which have an unusually reactive serine in their active site. Unfortunately, there are only a limited number of such mechanism-based enzyme-probe pairs, and so most of the proteome has been beyond the reach of ABPP.

To begin to move beyond this limitation, Weerapana *et al.*¹ turned to modestly reactive

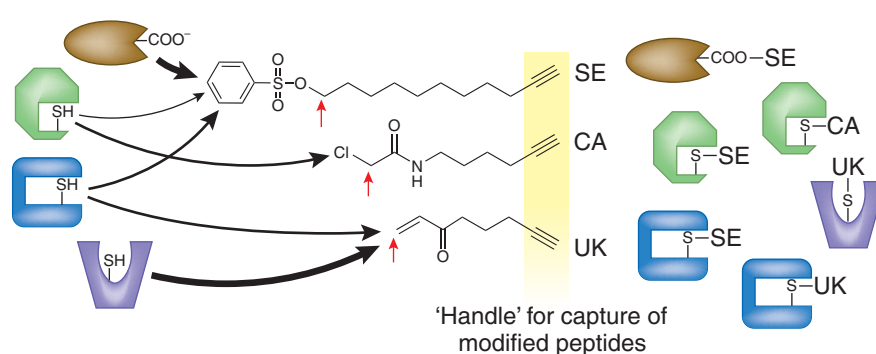


Figure 1 Second-generation ABPP chemistry. Three different electrophiles—a sulfonate ester (SE), a chloroacetamide (CA) and an unsaturated ketone (UK)—are used to modify the mouse liver proteome. UK and CA are cysteine-specific reagents for proteins, but they react with different cysteine-containing proteins. SE is more promiscuous and reacts with a number of protein residues. The result is that each reagent labels a different set of proteins, though there is some overlap in the reactivities. The red arrows indicate the electrophilic carbons. The black arrows indicate reactive partners, and their thickness indicates reaction rate. Each electrophile has an alkyne moiety to facilitate capture of the modified protein by click chemistry.

electrophiles (see Fig. 1). There is a long history in protein chemistry of using strongly electrophilic reagents to modify specific amino acid residues. For example, α -iodoacetamide will react with most surface-exposed cysteines in a protein. The goal here, however, was to ‘tone down’ the reactivity of the reagent such that one might observe differential reactivity based on the microenvironment of the nucleophilic amino acid in a particular protein, thereby making the molecule far more useful as an ABPP reagent.

Preliminary experiments revealed that epoxide-containing molecules are too unreactive to be of utility, whereas an unsaturated ketone (UK) was quite reactive and an α -chloroacetamide (CA) and sulfonate ester (SE) displayed more moderate protein reactivity. Experiments using simple amino acid derivatives showed that SE and especially CA were both quite selective for coupling to cysteine thiols, whereas UK was more promiscuous,

forming adducts with cysteine, histidine and lysine. Interestingly however, when these three reagents were incubated with a mouse liver extract, quite different results were obtained. Using a powerful analytical methodology that allowed specific capture of the modified peptide and the determination of the site of reaction of the electrophile on a protein, it was shown that CA and UK are quite specific for coupling to cysteines, whereas SE coupled to a variety of residues, especially glutamates. Moreover, an analysis of the identity of proteins modified by CA and UK in the liver extract showed that they are largely nonoverlapping. Taken together, these data argue strongly that the reactivities of electrophilic agents in the context of intact proteins can be very different than those exhibited for simple amino acids. Moreover, it demonstrates that the UK and CA probes, when applied to the proteome, are highly cysteine-selective modifiers that are highly sensitive to the microenvironment of the cysteine.

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This work demonstrates that modestly reactive nucleophiles can be useful ABPP reagents for proteins that one might not have predicted, *a priori*, to be targets. However, much more work remains to be done beyond this proof-of-principle study to determine how useful this second-generation ABPP strategy might be in 'spreading out' the proteome, particularly in comparison to other methods such as protein capture arrays⁴. In this report, only a few hundred chemically modified peptides were detected. This is still a tiny fraction of the

proteome. The good news is that it should be possible to decorate these simple molecules with substituents that modify their steric or electronic properties so as to create a much larger collection of electrophiles with different protein reactivities. One could then imagine incubating each with a complex proteome and pulling out for subsequent analysis a different set of proteins in each case. Moreover, the simple structures of these molecules will make it straightforward to synthesize isotopically labeled electrophiles to facilitate quantitative

comparisons between the levels of reactive proteins in two different samples⁵. Therefore, it seems likely that more elaborate ABPP-based techniques will fill a useful niche in the arsenal of proteome analysis methods.

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Unraveling a molecular target of macrolides

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Leucascandrolide A and neopeltolide are structurally related natural products with potent growth inhibitory activity. The synthesis of a designed analog of leucascandrolide A and its evaluation in a yeast haploinsufficiency screen has revealed the cytochrome *bc*₁ complex as a molecular target of these compounds.

It has been estimated that up to 50% of modern prescription drugs are derived from naturally occurring substances¹. Although the biological activity of natural products is often discovered in phenotypic cellular assays, the identification of their molecular targets generally represents an important prerequisite for their exploitation in drug discovery and development. This process can be hampered by a lack of material, which is notoriously problematic for marine natural products. In such cases synthesis provides the sole means to enable biological studies. Leucascandrolide A (**Fig. 1**) was discovered in 1996 and was found to exhibit potent antiproliferative activity against human cancer cells and the pathogenic yeast *Candida albicans*². Yet the molecular target of this natural product has remained unknown. On page 418 of this issue, Ulanovskaya *et al.*³ describe the chemical synthesis of a simplified analog of leucascandrolide A, which has enabled the identification of the mitochondrial cytochrome *bc*₁ complex as a principal molecular target of this natural product. These findings could then be extended to the structurally related marine macrolide neopeltolide (**Fig. 1**), which has been isolated only very recently

and shows a similarly promising biological activity profile⁴.

The key breakthrough from the Kozmin study stems from the hypothesis that neopeltolide might be considered a simplified analog of leucascandrolide A. The structural overlap along with their reported activity suggested the possibility that similar mechanisms were responsible for the inhibition of cellular proliferation by the two compounds. The story commences with the evaluation of biological activity for each of the two enantiomers of leucascandrolide, which are accessed by separation of the racemate produced from the first synthesis route⁵. The fact that these were close in activity along with the structural similarity between leucascandrolide A and neopeltolide suggested that the oxazole domain dominates the biological activity. This led to the design of the simplified chimeric structure **3** as a target for synthesis and biological studies.

The transition from the parent natural product to this simplified analog involved two key structural changes: the excision of the C12 and C21 methyls (**Fig. 1**). Although removal of the latter is nominally simplifying, it is deletion of the former that substantively impacts the synthesis strategy, allowing an efficient 24-step approach to the designed target. It is well worth noting that even the best intentioned design must ultimately be reduced to practice. The investigators had the advantage of a clever route that relies on the application of a Prins desymmetrization reaction⁵. Its implementation provided

efficient access to the pyran subunit, whose configuration serves as the keystone of the subsequent diastereoselective route³. Another unusual step stems from the observation of a surprisingly stable macrocyclic hemiketal that can be oxidized to furnish the targeted macrolactone. A related synthesis sequence was crafted for neopeltolide. Intriguingly, the chimeric simplified analog **3** shows activity that is equal to that of leucascandrolide A. Thus, an important lesson to be learned from this work, in hand with other examples in the recent literature⁶, is that chemical synthesis and biological profiling of simplified analogs can be of significant value in natural product-based drug discovery.

With analog **3** in hand as an enabling tool, the authors began a search for the molecule's mechanism of action. The key step in this process was the screening of **3** against a library of 4,900 yeast strains with different haploid nonessential gene deletions, which highlighted the *SNF4* gene, among others, as important⁷. The *SNF4* gene encodes a regulatory subunit of the yeast homolog of the stress-responsive mammalian AMP-activated kinase (AMPK), whose activation is partly triggered by inhibition of ATP production or stimulation of ATP consumption. The pronounced sensitivity of an *SNF4* deletion mutant to treatment with **3** in the presence of galactose indicated that **3** might target mitochondrial oxidative phosphorylation as the non-glucose-using pathway for ATP biosynthesis.

This insight led to follow-up experiments in yeast and mammalian cells, which secured the

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