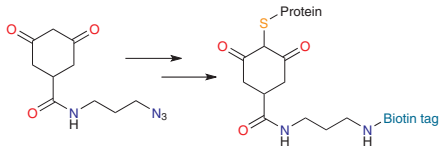
Sensing SAH selectively

Biological methylation reactions typically involve the transfer of the methyl group from the sulfonium group of (*S*)-adenosyl-L-methionine (SAM) to a target molecule, resulting in the generation of (*S*)-adenosyl-L-homocysteine (SAH). SAH inhibits many SAM-dependent enzymes and thus can be toxic at high concentrations. In bacteria, there are two pathways for recycling SAH back to SAM. While conducting a bioinformatic search for new classes of riboswitches, Wang *et al.* discovered a candidate riboswitch that was associated with genes involved in one of the SAH salvage pathways and that could therefore be a SAH-responsive regulator. This riboswitch was found to bind SAH tightly *in vitro* and to regulate the SAH-dependent expression of reporter genes *in vivo*. The riboswitch bound SAH at least 1,000-fold more tightly than SAM and most other SAH analogs. Consistent with the observed tight binding and high selectivity, virtually every functional group of SAH was important for recognition by the riboswitch. Only the removal of a methylene group was well tolerated, which suggests that the RNA aptamer might contain two separate binding pockets with some flexibility in their positioning. Four riboswitch classes have been identified that recognize SAM with two to three orders of magnitude selectivity over SAH. With the identification of a SAH-selective riboswitch, the RNA features controlling specificity for different adenosyl-containing ligands can now be determined, which may aid in the design of new biosensors. (*Mol. Cell* **29**, 691–702, 2008) *JK*

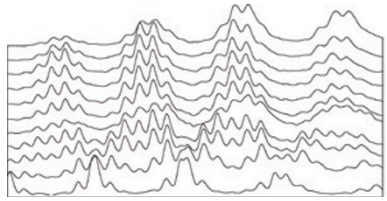
Sulfenic acids bedazzled



Reactive oxygen species (ROS) have been implicated in oxidative damage to cells, but they also have roles in cellular signaling, wherein reversible oxidation of proteins can modulate protein function. For example, post-translational modification of a cysteine to a sulfenic acid (Cys-SOH) can interfere with its role as a catalytic nucleophile in enzymes such as protein tyrosine phosphatases. Determining the role of cysteine oxidation in cells requires tools to profile the abundance of specific cysteine 'oxoforms' under physiological conditions. Reddie *et al.* now report a strategy for the capture and detection of sulfenic acid–modified proteins within cells. They synthesized a bifunctional tag, called DAz-1, which contains a dimedone moiety designed to react with sulfenic acids, tethered to an azide group, which provides a handle for postcapture labeling. Covalent protein modification by DAz-1 was demonstrated in the test tube for two model sulfenic acid–modified proteins. After DAz-1 labeling, the modified proteins were tagged with biotin via the azide handle of DAz-1 by Staudinger ligation. The authors further demonstrated using ESI-MS and western blotting that DAz-1 could be used to chemoselectively tag sulfenic acid–modified proteins from a mixture of proteins *in vitro* and also directly in Jurkat cells. This new profiling tool may provide new insights into the abundance and biological significance of these post-translational modifications. (*Mol. Biosystems*, published online 14 March 2008, doi:10.1039/b719986d) *TLS*

Written by Mirella Bucci, Catherine Goodman, Joanne Kotz & Terry L Sheppard

The fast and the complex


Carol Robinson

Determining the structure and dynamics of noncovalent protein complexes is important for a complete understanding of their function. Nanoflow electrospray ionization (nESI) combined with mass spectrometry (MS) has been used to monitor assembly and dynamics of protein complexes, but it is not ideal for real-time monitoring of macromolecular complexes and reaction mixtures, because early time points are missed. Now, Painter *et al.* use a robotic nESI chip platform with MS that allows for in-tandem sampling of multiple reaction mixtures that differ in their components or are separated in time. The authors first used this technique to examine the tryptic digestion of cytochrome *c* (CytC). The results were highly reproducible compared to continuous sampling and the resulting CytC decay kinetics and temperature-dependent rate constants were consistent with previous reports of this and other enzymatic digestion reactions. In addition, one species ($CytC_{1–79}$) was only found at intermediate time points, which demonstrates the advantage of the real-time approach over studies performed at equilibrium. Applying the rapid, automated nESI-MS approach to two small heat shock proteins, the authors found that both exist as noncovalent dodecamers that undergo rapid sequential exchange of dimeric units. The structural and dynamic details that can be obtained using the nESI-MS approach should prove useful for studying other subunit exchange reactions and other nonequilibrium protein complex states which are difficult to study using more conventional structural biology techniques. (*Chem. Biol.* **15**, 246–253, 2008) *MB*

Chemical shocks to OXPHOS

As the biological machinery for ATP synthesis, the oxidative phosphorylation (OXPHOS) system in mitochondria plays a pivotal role in energy homeostasis. Dysfunction of OXPHOS has severe consequences, but gaining mechanistic insight into this system has been challenging. Now Wagner *et al.* examine the effect of nearly 2,500 small molecules on the biology of OXPHOS. The authors compile the results of cell-based assays, such as measurements of mitochondrial membrane potential and reactive oxygen species (ROS), and gene expression information for 25 nuclear and mitochondrial OXPHOS proteins. Analysis of these data shows that, although the expression of the nuclear and mitochondrial genes is normally highly coordinated, some protein synthesis inhibitors such as cycloheximide were able to decouple these processes, which suggests that protein translation facilitates coordination. Additionally, three of six statins—compounds that inhibit cholesterol synthesis and are associated with myopathy—caused a unique response that was reiterated by other compounds associated with myopathy, thereby potentially establishing a signature for this drug-induced toxicity. These effects were closely matched with those of two electron transport chain inhibitors, strengthening the connection between myopathy and blockages in electron transport. Finally, two strategies for identifying compounds that elevate OXPHOS expression but decrease ROS accumulation highlighted an unusual link between microtubule modulators and OXPHOS behavior. Further investigation of these biological topics and future analysis of this rich data set will undoubtedly help to elucidate the detailed workings of this complex system. (*Nat. Biotech.* **26**, 343–351, 2008) *CG*