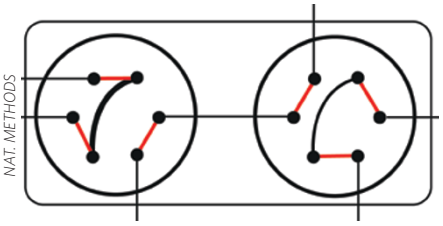
METABOLOMICS

## Budgeting on a diet

*Nat. Methods* doi:10.1038/nmeth.3584


NAT. METHODS

Gram-negative bacteria such as *Escherichia coli* respond to decreased levels of carbon by entering a stationary phase associated with increased protein degradation and decreased synthesis. Although mass spectrometry approaches have identified changes in a number of metabolites during starvation, this process requires constant extraction of metabolites and MS analysis, which can be technically demanding. Link *et al.* developed a method that allows the real-time monitoring of 300 compounds in live cells ranging from bacteria to mammalian cells at 15- to 30-second intervals. A cultivation vessel was attached to a time-of-flight (TOF) mass spectrometer, which ensures the constant cycling and sampling of cells through direct injection into the spectrometer. This approach produces findings comparable to those of an established manual metabolomics approach but has much higher temporal resolution. The authors observed metabolic dynamics in *E. coli* at different time points during the two hours of starvation and 30 minutes after adding glucose. They focused on two clusters of metabolites whose levels changed during the analysis: a group of metabolically costly molecules such as valine and tryptophan that accumulated during starvation and decreased after glucose addition and a second group of energetically cheaper compounds such as succinate that were scarce during starvation and more abundant under fed conditions. The authors proposed that the different dynamics in these two groups were due to changes in amino acid degradation. Mathematical modeling using the profiling data coupled with protein synthesis inhibitor experiments revealed that these changes were due to feedback inhibition of the biosynthesis of the costly amino acids resulting in the utilization of the cheaper amino acids for protein synthesis. Overall, the utility of this method to provide real-time analysis of the metabolic status of a cell may reveal new potential regulatory mechanisms under different environmental conditions. *GM*

RNA PROFILING

## A better miR trap

*Mol. Cell.* **59**, 858–866 (2015)

Bioorthogonal chemistry approaches, which often include metabolic tagging with a chemical handle and its subsequent modification using a chemoselective reaction, have made it possible to specifically label biomolecules in cells. Many of these tools focus on protein labeling, but methods for tagging other biomolecules have enabled technologies including 'omic-'scale analysis of cellular nucleic acids. One approach for the reversible isolation of cellular RNAs involves metabolic labeling of transcripts with 4-thiouridine ($s^4U$) nucleotides and selective disulfide formation between thiolated bases and a biotin affinity tag. Duffy *et al.* now report an enhancement of this method that allows them to robustly track microRNA (miRNA) turnover within cells. To optimize the disulfide exchange reaction between the $s^4U$-modified RNA and the capture reagent, the authors used a methylthiosulfonate-activated form of biotin (MTS-biotin) and demonstrated that it outperformed the original reagent, efficiently modifying $s^4U$ nucleotides with high yields and in short reaction times. Profiling metabolically labeled RNAs from HEK293T cells with MTS-biotin revealed that the reagent provided broader coverage of the transcriptome and an enhanced representation of short RNA sequences than the earlier protocol. The greater efficiency of MTS-biotin enabled the authors to measure the levels of miRNAs in HEK293T cells without perturbing transcription or miRNA biogenesis pathways. Time-dependent labeling and deep sequencing revealed changes in cellular miRNA abundance, confirmed the existence of a subset of known miRNAs with fast-turnover kinetics and further identified two miRNAs whose turnover rates could not be characterized by other methods. The chemical insights of the current study have substantially improved this $s^4U$-based RNA profiling method, enhancing its utility for future explorations of RNA dynamics in cells. *TLS*

METALLOENZYMES

## A tungsten triple play

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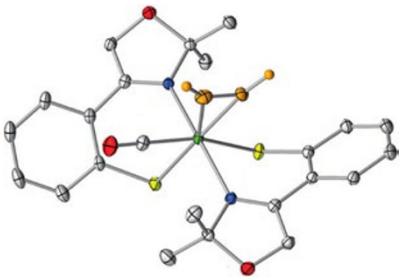
ANGEW. CHEM. INT. EDN. ENGL.

Acetylene hydratase catalyzes the conversion of acetylene ($C_2H_2$) to acetaldehyde ($CH_3CHO$) via an ethenol ($CH_2CHOH$) intermediate. Most of the proposed mechanisms for this tungsten-based enzyme require activation of acetylene, but the details of the process and the remaining steps in the catalytic pathway are controversial. The development of a biomimetic organometallic complex that could be used as a proxy to understand the tungsten (W) active site would help to shed light on the reaction, but only a few W-$C_2H_2$ complexes have been reported, and these either were intended to explore other questions or were not stable enough to interrogate. Peschel *et al.* report a new complex that includes two bidentate thiol-containing ligands, termed S-Phoz, meant to approximate the stability provided by the five sulfur ligands seen in the crystal structure of the enzyme. Exposure of a $W(CO)_2$(S-Phoz)$_2$ complex to an acetylene atmosphere led to the formation of a stable W-$C_2H_2$ adduct (structure shown). An oxo donor led to the further displacement of the remaining CO ligand by an oxygen, yielding $WO(C_2H_2)$(S-Phoz)$_2$. Exposure of this complex to light led to the rapid dissociation of the acetylene group, creating a third unique species, whereas incubation under dark conditions led to the slow reattachment of the acetylene, providing a rare example of the reversible activation of acetylene. Crystal structures of these three forms demonstrated that upon coordination, the linear $C_2H_2$ becomes bent, with longer carbon-carbon triple bonds, both indicative of activation. The asymmetric coordination of $C_2H_2$ also suggests an asymmetric charge distribution, providing glimpses into possible points of attack for an incoming nucleophile. This system provides a much-needed scaffold to inform further mechanistic studies of acetylene hydratase. *CG*