LABELING

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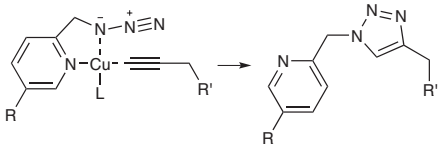
Angew. Chem. Int. Ed. Engl., published online 3 May 2012, doi:10.1002/anie.201108181



First-generation azide-alkyne cycloaddition reactions required high doses of copper, the toxicity of which limited cellular applications. Next-generation reactions have thus focused on decreasing the copper required by altering metal ligands or by using ring strain to drive the reaction. Uttamapinant *et al.* now present a third strategy: raising the effective copper concentration by merging a copper chelator with the substrate azide. To test this idea, the authors synthesized substrates containing pyridine rings with nitrogen atoms positioned next to the reactive azide; these substrates showed 40–80% conversion to product in 30 minutes, whereas control phenyl-modified substrates showed no conversion in that time. Adding the known copper ligand THPTA increased conversion rates, but not as much as chelation, as a pyridine-modified substrate without THPTA was equally or more effective than a phenyl-modified substrate with THPTA. To extend this reaction to cells, the authors used their PRIME strategy to specifically link azides onto protein fusions prior to cycloaddition. Rates of protein labeling mirrored *in vitro* results, with chelation increasing yields by 2.7- to 25-fold. The combined use of chelation and secondary copper ligands allowed the use of copper at sufficiently low concentrations that proteins could be labeled without disrupting neuron cultures known to be highly sensitive to toxic substances, suggesting that this strategy will find immediate utility in chemical biology research. *CG*

NEUROBIOLOGY

Metabolites tempt fate

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Under stressful conditions, such as when glucose is limited, neurons undergo a cytoprotective program where anaerobic biosynthesis of lactate leads to changes in neuronal membrane potential and cell signaling. Because suitable analytical tools to monitor dynamic stress responses are scarce, the identity of key metabolic mediators of this neuronal stress response remain unknown. To identify essential metabolic events that mediate injury, McKenzie *et al.* now use multianalyte microphysiometry to measure changes in lactate release, oxygen consumption and acidification rates after periods of glucose deprivation in neuronal cultures. Oxygen consumption was irreparably affected in pure neuronal cultures but was not affected when glia cells were mixed with the neurons, consistent with a supporting role of glia cells in the brain. Acidification decreased in both cultures, but the magnitude decrease was greater in the mixed cultures and the levels were not fully recovered upon readdition of glucose. Also, there was an increase in cell death in the neuronal but not the mixed cultures. Taken together and with subsequent experiments to measure oxidative modification of proteins and lipids during glucose deprivation, these findings suggest that extracellular acidification and oxygen consumption were the best predictors of neuronal survival in the presence of glia, correlating better than current clinical metrics of tissue damage, such as lactate concentrations and lipid oxidation. *MB*

SYSTEMS BIOLOGY

Unmasking death pathways

Cell **149**, 780–794 (2012)

New strategies to treat triple-negative breast cancers (TNBCs), which lack estrogen receptor or progesterone receptor expression and amplification of *HER2*, are needed. To determine whether manipulation of growth factor signaling can influence the sensitivity of TNBCs to DNA-damaging agents, Lee *et al.* combined genotoxic drugs with small molecules that target oncogenic pathways and compared outcomes in TNBC cells to those in other breast cancer cell lines. Combinations in which the EGFR inhibitor erlotinib was added to cells for several hours before addition of the genotoxic agent enhanced apoptosis. The authors validated that the increase in apoptosis resulted from the on-target activity of erlotinib by using other EGFR inhibitors and short interfering RNA (siRNA) knockdown of EGFR. After ruling out changes in cell cycle progression, drug influx or efflux and changes in DNA damage, they showed that prolonged EGFR inhibition suppressed a Ras oncogene expression signature to enhance DNA damage-induced cell death. The authors used a multiplex systems biology approach to quantify 35 signaling proteins at various time points after treatment with erlotinib, genotoxic agent or both; an iterative mathematical modeling analysis of these data revealed that caspase-8 was the most important mediator of apoptosis. siRNA knockdown confirmed that caspase-8 was critical for the apoptotic response. These data indicate that pretreatment but not co-treatment of some TNBCs with EGFR inhibitors can rewire signaling networks and unmask proapoptotic pathways, thereby synergizing with genotoxic agents to induce apoptosis. *AD*

DRUG DISCOVERY

Killing amoebas

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Entamoeba histolytica, an intestinal protozoan parasite, is the causative agent of the life-threatening infection amebiasis. Debnath *et al.* now identify auranofin, a US Food and Drug Administration–approved drug, as a potent inhibitor of *E. histolytica* growth. The authors screened the 910-member Iconix library and identified 11 compounds with strong amoebicidal activities, with auranofin being the most potent of all. Although auranofin has been clinically used for treating rheumatoid arthritis for years, its mode of action has remained elusive. To gain insight into the molecular mechanism, the authors compared the gene expression signatures of untreated and auranofin-treated parasites. The arsenite-inducible RNA-associated protein transcript was significantly upregulated in auranofin-treated parasites, leading the authors to hypothesize that arsenite and auranofin target the same protein in *E. histolytica*. Previous work had suggested that both arsenite and auranofin inhibit thioredoxin reductase (TrxR), an enzyme involved in reactive oxygen species detoxification. The authors showed that auranofin could inhibit the enzymatic activity of recombinant *E. histolytica* TrxR *in vitro*, whereas *in vivo* auranofin rendered the trophozoites highly susceptible to H_2O_2. Also, the drug inhibited the infection in rodent models of amebiasis. In sum, the authors, by screening a high-throughput library, repurposed auranofin and showed that it can target the growth of *E. histolytica* and could potentially be used in treating amebiasis. *AC*